Fronda, Christian

From: Fronda, Christian
Sent: Wednesday, August 06, 2003 10:28 AM
T : STIC-Biotech/ChemLib
Subject: Non patent literature - Serial No. 09/914,006

Importance: High

I would like to order the following non patent literature listed below. Thank you.

Christian Fronda
Art Unit 1652
Mailbox CM1 10D01
Office CM1 11E03
(703)305-1252

L14 ANSWER 2 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:961953 HCAPLUS
DOCUMENT NUMBER: 124:77988
TITLE: Cloning, sequencing and expression of the ilvBNC gene cluster from Streptomyces avermitilis
AUTHOR(S): De Rossi, Edda; Leva, Raffaella; Gusberti, Laura; Manachini, Pier Luigi; Riccardi, Giovanna
CORPORATE SOURCE: Department of Genetics and Microbiology, University of Pavia, 27100, Pavia, Italy
SOURCE: Gene (1995), 166(1), 127-32
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

L14 ANSWER 5 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:1848 HCAPLUS
DOCUMENT NUMBER: 120:1848
TITLE: Isoleucine synthesis in Corynebacterium glutamicum: molecular analysis of the ilvB-ilvN-ilvC operon
AUTHOR(S): Keilhauer, Carmen; Eggeling, Lothar; Sahm, Hermann
CORPORATE SOURCE: Inst. Biotechnol., Forschungszent., Juelich, D-52425, Germany
SOURCE: Journal of Bacteriology (1993), 175(17), 5595-603
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

L14 ANSWER 5 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1994:1848 HCAPLUS
DOCUMENT NUMBER: 120:1848
TITLE: Isoleucine synthesis in Corynebacterium glutamicum: molecular analysis of the ilvB-ilvN-ilvC operon
AUTHOR(S): Keilhauer, Carmen; Eggeling, Lothar; Sahm, Hermann
CORPORATE SOURCE: Inst. Biotechnol., Forschungszent., Juelich, D-52425, Germany
SOURCE: Journal of Bacteriology (**1993**), 175(17), 5595-603
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Acetohydroxy acid synthase** (AHAS) and **isomeroreductase** (IR) catalyze subsequent reactions in the flux of metabolites towards isoleucine, **valine**, leucine, and pantothenate. A 4705-bp DNA fragment from Corynebacterium glutamicum known to code for AHAS and IR was sequenced and analyzed by Northern (RNA blot) anal. As in other bacteria, the AHAS of this gram-pos. organism is encoded by two genes, ilvB and ilvN. Gene disruption verified that these genes encode the single AHAS activity in C. glutamicum. The start of ilvB was detd. by amino-terminal sequencing of a fusion peptide. By Northern anal. of the ilvBNC cluster, three in vivo transcripts of 3.9, 2.3, and 1.1 kb were identified, corresponding to ilvBNC, ilvNC, and ilvC messages, resp. The ilvC transcript (encoding IR) was by far the most abundant one. With a clone from which the ilvB upstream regions had been deleted, only the ilvNC and ilvC transcripts were synthesized, and with a clone from which the ilvN upstream regions had been deleted, only the smallest ilvC transcript was formed. It is therefore concluded that in the ilv operon of C. glutamicum, three promoters are active. The amts. of the ilvBNC and ilvNC transcripts increased in response to the addn. of .alpha.-ketobutyrate to the growth medium. This was correlated to an increase in specific AHAS activity, whereas IR activity was not increased because of the relatively large amt. of the ilvC transcript present under all conditions assayed. Therefore, the steady-state level of the ilvBNC and ilvNC messages contributes significantly to the total activity of the single AHAS. The ilvC transcript of this operon, however, is regulated independently and present in a large excess, which is in accord with the const. IR activities detd.

102b does PRIOR?
ilvBN
ilvC
claim 2

IL

L14 ANSWER 2 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1995:961953 HCAPLUS
DOCUMENT NUMBER: 124:77988
TITLE: Cloning, sequencing and expression of the ilvBNC gene cluster from Streptomyces avermitilis
AUTHOR(S): De Rossi, Edda; Leva, Raffaella; Gusberti, Laura; Manachini, Pier Luigi; Riccardi, Giovanna
CORPORATE SOURCE: Department of Genetics and Microbiology, University of Pavia, 27100, Pavia, Italy
SOURCE: Gene (**1995**), 166(1), 127-32
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The metab. of the branched-chain amino acids (BCAA) isoleucine, leucine and **valine** is correlated to the prodn. of polyketide antibiotics in many streptomycetes. Despite its significance, this biosynthetic pathway is poorly understood in Streptomyces. In order to develop a better understanding of Streptomyces BCCA biosynthesis, two genes, ilvBN and ilvC, encoding **acetohydroxy acid synthase** (AHS) and **acetohydroxy acid isomeroreductase** (IR), resp., were cloned from Streptomyces avermitilis, a strain producing avermectins, potent antiparasitic compds. The genes were isolated by applying a combination of PCR and genomic library screening. The deduced amino-acid sequences revealed significant homol. to the AHS and IR proteins from other bacterial species. The ilvBN gene, expressed in Escherichia coli (Ec) by using the expression vector pGEX-4T-1, complemented the ilv- mutation of Ec PS1283. Ec transformants produced high levels of AHS, whose activity was feedback inhibited by **valine**.

STIC-ILL

From: STIC-Biotech/ChemLib
Sent: Wednesday, August 06, 2003 10:40 AM
To: STIC-ILL
Subject: FW: Non patent literature - Serial No. 09/914,006

Importance: High

-----Original Message-----
From: Fronda, Christian
Sent: Wednesday, August 06, 2003 10:28 AM
To: STIC-Biotech/ChemLib
Subject: Non patent literature - Serial No. 09/914,006
Importance: High

I would like to order the following non patent literature listed below. Thank you.

Christian Fronda
Art Unit 1652
Mailbox CM1 10D01
Office CM1 11E03
(703)305-1252

L14 ANSWER 2 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:961953 HCAPLUS
DOCUMENT NUMBER: 124:77988
TITLE: Cloning, sequencing and expression of the ilvBNC gene cluster from Streptomyces avermitilis
AUTHOR(S): De Rossi, Edda; Leva, Raffaella; Gusberti, Laura; Manachini, Pier Luigi; Riccardi, Giovanna
CORPORATE SOURCE: Department of Genetics and Microbiology, University of Pavia, 27100, Pavia, Italy
SOURCE: Gene (1995), 166(1), 127-32
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

L14 ANSWER 5 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:1848 HCAPLUS
DOCUMENT NUMBER: 120:1848
TITLE: Isoleucine synthesis in Corynebacterium glutamicum: molecular analysis of the ilvB-ilvN-ilvC operon
AUTHOR(S): Keilhauer, Carmen; Eggeling, Lothar; Sahm, Hermann
CORPORATE SOURCE: Inst. Biotechnol., Forschungszent., Juelich, D-52425, Germany
SOURCE: Journal of Bacteriology (1993), 175(17), 5595-603
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

Gene, 166 (1995) 127–132
 0378-1119/95/$09.50

GENE 09277

Cloning, sequencing and expression of the *ilvBNC* gene cluster from *Streptomyces avermitilis*

(Branched-chain amino-acid biosynthesis; polymerase chain reaction; avermectin; *Escherichia coli*)

Edda De Rossi[a], Raffaella Leva[a], Laura Gusberti[a], Pier Luigi Manachini[b] and Giovanna Riccardi[a]

[a]*Department of Genetics and Microbiology, University of Pavia, 27100 Pavia, Italy; and* [b]*Department of Science and Microbiological Technologies, University of Milano, via Celoria 2, 20133 Milano, Italy. Tel. (39-2) 2666-160*

Received by K.F. Chater: 18 April 1995; Revised/Accepted: 29 June/30 June 1995; Received at publishers: 18 August 1995

SUMMARY

The metabolism of the branched-chain amino acids (BCAA) isoleucine, leucine and valine is correlated to the production of polyketide antibiotics in many streptomycetes. Despite its significance, this biosynthetic pathway is poorly understood in *Streptomyces*. In order to develop a better understanding of *Streptomyces* BCCA biosynthesis, two genes, *ilvBN* and *ilvC*, encoding acetohydroxy acid synthase (AHS) and acetohydroxy acid isomeroreductase (IR), respectively, were cloned from *Streptomyces avermitilis*, a strain producing avermectins, potent antiparasitic compounds. The genes were isolated by applying a combination of PCR and genomic library screening. The deduced amino-acid sequences revealed significant homology to the AHS and IR proteins from other bacterial species. The *ilvBN* gene, expressed in *Escherichia coli* (*Ec*) by using the expression vector pGEX-4T-1, complemented the *ilv*$^-$ mutation of *Ec* PS1283. *Ec* transformants produced high levels of AHS, whose activity was feedback inhibited by valine.

INTRODUCTION

Streptomyces spp. are Gram$^+$ soil bacteria that show characteristic morphological differentiation as well as physiological differentiation (Chater, 1993). In many streptomycetes, BCAA regulatory mutants show changes in production levels of polyketide antibiotics (Pospisil et al., 1984). It was therefore of interest to study the organization of the *ilv* genes in *S. avermitilis* (*Sa*), a strain producing avermectins, antiparasitic compounds with broad-spectrum activity against nematode and arthropod parasites. The avermectin molecule is composed of aglycone and disaccharide moieties. The carbon skeleton of the avermectin aglycone originates from 7 malonyl-CoAs, 5 methylmalonyl-CoAs and 1 molecule of either 2-methylbutyryl-CoA or isobutyryl-CoA as starter unit. Malonyl- and methylmalonyl-CoAs are considered to be formed from the common intermediates of primary

Correspondence to: Dr. G. Riccardi, Department of Genetics and Microbiology, via Abbiategrasso 207, 27100 Pavia, Italy. Tel. (39-382) 505-574; Fax (39-382) 528-496; e-mail: Riccardi@ipvgen.unipv.it

Abbreviations: aa, amino acid(s); Ab, antibody(ies); AHS, acetohydroxy acid synthase; Ap, ampicillin; BCAA, branched-chain aa; bp, base pair(s); CoA, coenzyme A; *Cg*, *Corynebacterium glutamicum*; dATP, deoxyadenosine triphosphate; DTT, dithiothreitol; *Ec*, *Escherichia coli*; FAD, flavin adenine dinucleotide; GST, gluthathione-*S*-transferase; *ilvA*, gene encoding threonine deaminase; *ilvBN*, gene encoding AHS; *ilvC*, gene encoding IR; *ilvD*, gene encoding dihydroxy acid dehydratase; *ilvE*, gene encoding branched-chain aa aminotransferase; IPTG, isopropyl-β-D-thiogalactopyranoside; IR, acetohydroxy acid isomeroreductase; kb, kilobase(s) or 1000 bp; *leuACBD*, genes encoding α-isopropylmalate synthase, isopropylmalate isomerase and β-isopropylmalate dehydrogenase, respectively; nt, nucleotide(s); oligo, oligodeoxyribonucleotide; ORF, open reading frame; PAGE, polyacrylamide-gel electrophoresis; PCR, polymerase chain reaction; RBS, ribosome-binding site(s); re-, recombinant; *S.*, *Streptomyces*; *Sa*, *S. avermitilis*; SDS, sodium dodecyl sulfate; TTP, thiaminepyrophosphate; [], denotes plasmid-carrier state.

SSDI 0378-1119(95)00563-3

metabolism; hence the synthesis of the branched-chain precursors is probably of key importance with respect to the subsequent formation of the secondary metabolite. 2-methylbutyryl-CoA, the building unit for biosynthesis of the predominating group of avermectins ('a'), is derived by the action of branched-chain 2-oxo acid dehydrogenase from 2-oxo-3-methyl-valerate arising either by degradation of isoleucine, or by de novo synthesis via the BCAA biosynthetic pathway (Hafner et al., 1991).

The first step in the BCAA pathway, leading specifically to isoleucine production, is the deamination of threonine to form α-ketobutyrate, a step mediated by the enzyme threonine deaminase. For the next four steps, the same enzymes catalyze the reactions leading to the formation of either isoleucine or valine, i.e., the reactions occur in parallel. First, an active acetaldehyde derived from pyruvate is transferred to α-ketobutyrate (isoleucine biosynthesis) or to another pyruvate (valine biosynthesis) by the enzyme acetohydroxy acid synthase (AHS, EC 4.1.3.18). The acetohydroxy acids are then converted to dihydroxy acids by acetohydroxy acid isomeroreductase (AHAIR, EC 1.1.1.86) and to branched-chain keto acids by the dihydroxy acid dehydratase. The final step involves a transamination of the branched-chain keto acids to isoleucine or valine.

In the present study, we describe the cloning of the *ilvBN* genes from *Sa*. Sequence analysis revealed the *ilvC* gene downstream from *ilvBN*. The putative *ilvBN* gene was found to complement an *Escherichia coli ilv*$^-$ mutation when expressed from an *Ec* promoter.

EXPERIMENTAL AND DISCUSSION

(a) Cloning and sequencing of the *ilvBNC* cluster from *Sa*

For cloning the entire AHS-encoding gene, a DNA library of *Sau*3AI fragments from *Sa*, constructed in *Ec* using λEMBL-3, was screened with a 972-bp amplified PCR product (see Fig. 1 for details) and three positive phages were obtained (λEMBL-3-T1, -T2, -T3). DNA from recombinant clones λEMBL-3-T1, T2 and T3 was digested with *Pvu*II and *Sal*I, blotted and hybridized with labelled PCR-fragment. The probe hybridized to a *Pvu*II fragment of about 3.7 kb and *Sal*I fragment of 2.2 kb that were subcloned into pGEM-4Z to yield pSa3.7 and pSa2.2, respectively. The 2.2-kb *Sal*I fragment was used as a probe to clone the 2.0-kb *Pvu*II fragment (Fig. 1).

The nt sequence of the fragment extending from the *Eco*RI site to the extreme *Pvu*II site was determined on both strands. PC/GENE analysis indicated that the fragment contains three ORFs: ORF1 (nt 439–2289), ORF2 (nt 2313–2840) and ORF3 (nt 2963–3961), transcribed in the same direction. All the ORFs are preceded by potential RBS which exhibit varying degrees of complementarity to the 3′ end of 16S rRNA from *S. lividans* (Suzuki and Yamada, 1988).

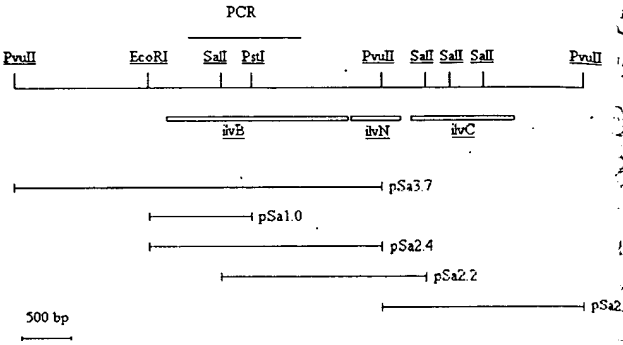


Fig. 1. Restriction map of the region containing the *ilvBNC* gene cluster from *Sa*. The clones used for sequencing are also shown. **Methods:** To amplify a *ilv*-specific fragment from genomic *Sa* CU18 (Cimburkova et al., 1988) DNA, two 23-mer oligos were designed from the aa sequences GPGATN and VGQHQM, which are highly conserved in AHS polypeptides. The nt sequence of the coding strand primer is 5′-TTGGATCCGGACCGGG(A,C,T)GCCACCAA (3-fold degeneracy); it has an extension of 8 nt at the 5′ end, six of which are a *Bam*HI site (underlined), and two T residues serve as a clamp for efficient cleavage by *Bam*HI after PCR amplification. The nt sequence of the complementary strand is 5′-TTGAGCTCCAT(C,T)TG(A,G)TGC-TGACC (4-fold degeneracy); it also has an extension of 8 nt at the 5′ end, six of which are a *Sac*I site (underlined), and two T residues serve as a clamp to facilitate cleavage by *Sac*I. As a template, *Sa* genomic DNA was heat denaturated (2 min at 94°C) prior to amplification. PCR was performed with a 40-μl mixture of 10 mM Tris·HCl pH 8.3/50 mM KCl/1.5 mM $MgCl_2$/200 μmol each of dNTP/100 pmol of each primer/100 ng of template DNA/2.5 u of Ampli*Taq* polymerase (Perkin-Elmer Cetus). The temperature profile of the first three PCR cycles was 30 s at 94°C, 1 min at 42°C and 2 min at 72°C. After these initial cycles, the samples were subjected to another 27 cycles of 30 s at 94°C, 1 min at 60°C and 2 min at 72°C, followed by a further incubation at 72°C for 5 min. The PCR product was a band of approx. 1000 bp, including the extensions of the PCR primers; DNA from this band, extracted from 1.2% agarose gel, was digested with *Bam*HI + *Sac*I and cloned into *Bam*HI + *Sac*I-treated sequencing vector pGEM-4Z. Sequencing revealed that the insert encoded a 324-aa sequence with a good degree of homology with the central region of the AHS aa sequences, chosen for the design of the PCR primers.

The 1851-nt ORF1 has two potential translation start sites. The ATG at nt 439–441 is favoured because it is preceded by a better putative RBS and has better spacing than the alternative ATG (nt 433–435) (Fig. 2). ORF1 encodes a protein of 617 aa (65.9 kDa).

Alignment of the aa sequence revealed a high degree of identity, 60 and 62%, with sequences of AHS large subunits from *Corynebacterium glutamicum* (*Cg*) (Keilhauer et al., 1993) and *Caulobacter crescentus* (Tarleton et al., 1994), respectively. A lower identity was found with AHS sequences of *Ec* (44, 43, 41% identity with AHSI (Wek et al., 1985), AHSII (Lawther et al., 1987) and AHSIII (Squires et al., 1983), respectively), *L. lactis* (44%; Godon et al., 1992) and *B. subtilis* (48%; Vandeyar et al., 1986). ORF2 encodes a protein of 176

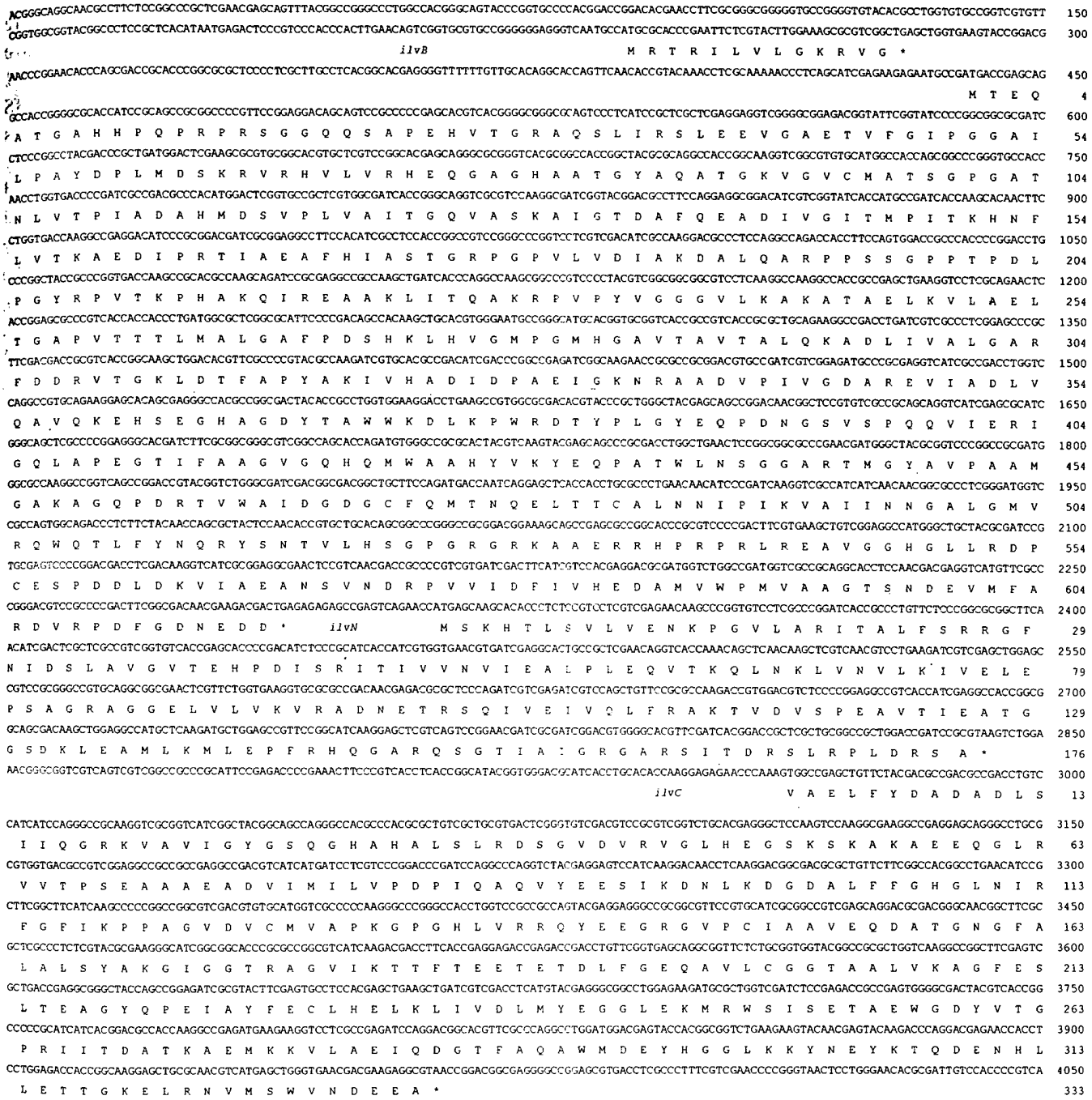


Fig. 2. Nucleotide sequence of the *Sa ilvBNC* cluster and deduced aa sequence. Underlined sequences are potential RBS. In front of *ilvB*, a possible leader peptide and a potential Rho-independent transcription termination signal are shown. A potential terminator after *ilvN* is shown too. The nt sequence reported here has been deposited in the GenBank Data Library under accession No. L39268. **Methods:** DNA inserts were digested with several restriction enzymes, and the resulting fragments were subcloned in pGEM-4Z. Sequencing was performed by the dideoxy chain-termination method (Sanger et al., 1977) using the Pharmacia T7 Polymerase Sequencing kit according the supplier's instructions with [α-^{35}S]dATP. The sequence was determined on both strands and all restriction sites used to subclone in pGEM-4Z plasmid were overlapped. DNA sequence analysis, assembly and editing of the data, analysis of the putative ORFs, codon usage and restriction sites of the different gene products, were performed using PC/GENE program.

aa with an estimated molecular mass of 19 124 Da, showing significant aa identity with the *ilvN* gene products of *Cg* (44%), *C. crescentus* (46%), *Ec* (31%) and *L. lactis* (30%) and to the *ilvH* (38%) and *ilvM* (26%) gene products of *Ec*, all encoding the small subunits of AHS. ORF1 and ORF2 were therefore named *ilvB* and *ilvN*, in accordance with the *Ec* and *B. subtilis* nomenclature. Thus, the AHS of *Sa* is encoded by two genes (*ilvB* and *ilvN*), as found for all other bacterial biosynthetic AHS enzymes (Umbarger, 1987). The proximity of the *ilvN*

gene to the translation stop codon of *ilvB* indicates that this gene may be cotranscribed. In all prokaryotic AHS-encoding genes characterized to date, the large and the small AHS subunits are encoded by adjacent genes in the same transcription unit (Umbarger, 1987), in contrast to the cyanobacterial AHS, which consist of a single polypeptide (Milano et al., 1992).

Downstream of *ilvN* is ORF3, encoding a product of 333 aa with a predicted molecular mass of 36 211 Da. A search of the databases revealed significant sequence identity between ORF3 and several bacterial IR, from *Cg* (62%; Keilhauer et al., 1993), *Rhizobium meliloti* (50%; Aguilar and Grasso, 1991), *B. subtilis* (57.6%; Fink, 1993), *L. lactis* (57%; Godon et al., 1992) and *Ec* (37%; Wek and Hatfield, 1986). The deduced molecular mass of *Sa* IR differs significantly from that reported for *Ec* IR (36.2 vs. 54.0 kDa) (Wek and Hatfield, 1986).

Examination of the nt sequence upstream from *ilvB* revealed a possible start codon at nt 240 (preceded by a potential RBS) that would result in a peptide of 13 aa. This ORF is followed by a sequence which resembles a *rho*-independent terminator. Similar ORFs encode leader peptides which function as part of an attenuation mechanism to regulate a number of prokaryotic biosynthetic operons (Yanofsky, 1987). Further studies are in progress to give an insight into the regulatory mechanisms of *Sa ilvBN* locus.

The BCAA pathway, by which leucine, isoleucine and valine are synthesized, has been widely studied in bacteria. Organization of the genes of the BCAA pathway has been characterized for *Ec* and *S. typhimurium* (Umbarger, 1987), *B. subtilis* (Vandeyar et al., 1986), *Cg* (Cordes et al., 1992) and *L. lactis* (Godon et al., 1992). In *B. subtilis ilvBNC* and *leuACBD* are found in one chromosomal region and *ilvAD* in another. Three non-contiguous chromosomal fragments cloned from *Cg* carry five BCAA genes: *ilvCBN*, *ilvA* and *ilvE*, while in *L. lactis* nine structural genes are clustered on a 12-kb fragment in the order *leuABCDilvDBNCA*. From our data, in *Sa* the organization of the BCAA genes seems similar to that in *Cg*. No other genes of the *ilv* cluster seem to be present in the regions up- and downstream the *ilvBNC* genes (data not shown), suggesting that the rest of the genes are scattered throughout the chromosome.

Genomic DNA was also analyzed in order to establish if one or more genes encoding AHS were present. The DNA was digested with several restriction endonucleases and blots were probed, using the 2.2-kb fragment from pSa2.2, at low stringency and washed at both low and high temperatures. In both cases, all the blots revealed the same banding patterns, thus indicating that there is a single copy of the AHS gene in the genome of *Sa*.

(b) Expression of the *Sa* re-AHS protein

In order to express the *Sa ilvBN* genes in *Ec*, they were subcloned into the expression vector pGEX-4T-1, to give pFP1. When expression was induced by IPTG, pFP1 complemented the *ilv*$^-$ mutation of *Ec* PS1283. The AHS activity was measured in various bacterial extracts (Table I). The PS1283 host strain had a specific activity of less than 0.0004 μmol/min per mg protein, as did PS1283[pGEX-4T-1]. In contrast, PS1283[pFP1] had a specific activity of 0.14 mmol/min per mg protein. For comparison, *Ec* PS1283[pTCN12], which carried the *ilvBN* gene, coding AHSI of *Ec* (Newman et al., 1982), had a specific activity of 0.2 mmol/min per mg protein. AHS activity was also assayed in the presence of 0.1 mM valine; in *Ec* PS1283[pFP1], specific activity was reduced about 50% compared with the control.

Total cell lysates of *Ec* cultures containing pGEX-4T-1 or pFP1 were prepared at various times from the start of IPTG induction and analyzed by SDS-PAGE. *Ec* cells transformed with pGEX-4T1 showed high-level expression of the 26-kDa GST (Fig. 3A, lane 3). The presence of a 92-kDa polypeptide (the predicted molecular mass of the *Sa* AHS protein fused to GST) is clearly evident from extracts of those PS1283[pFP1] (Fig. 3A, lane 4), whereas no such polypeptide is seen in those cells harboring the control plasmid (Fig. 3A, lane 3). Cleavage of this

TABLE I

Detection of AHS activity[a]

Plasmid	Specific activity[b]	Valine inhibition[c] (%)
No plasmid	0.0004	n.d.
pGEX-4T-1	0.0004	n.d.
pFP1	0.14	50
pTCN12	0.20	75

Methods: [a] All strains are *Ec* PS1283. Cells, grown to mid-exponential phase in minimal medium (Davis and Mingioli, 1950) were harvested and resuspended in 5 ml/g ice-cold extraction buffer (20 mM K·phosphate pH 7/20% glycerol/1 mM $MgCl_2$/0.25 mM DTT/0.1 mM TPP/0.01 mM FAD). The suspensions were sonicated (2 × 5 s), clarified by centrifugation (30 min at 20 000 × *g*) and the supernatants further centrifuged 90 min at 100 000 × *g*. The S-100 fractions were finally dialyzed against ice-cold extraction buffer and then stored at −20°C.

[b] AHS activity was assayed in a 0.2-ml reaction mixture containing 40 mM Na·pyruvate/10 mM $MgCl_2$/0.17 mM TPP/2.5 μM FAD/0.1 M KH_2PO_4 buffer pH 7.0. After 0.2 ml of extract was added, the mixture was incubated at 37°C for 60 min; the reactions were terminated by the addition of 0.04 ml 50% H_2SO_4, and then incubated at 37°C for 30 min. Colour development occurred with the addition of 0.4 ml of a 0.5% creatine hydrate solution and 0.4 ml of 5% α-naphthol (5 g in 100 ml 4 M NaOH). After brief vortexing, the reaction tubes were allowed to sit for 40 min at 37°C, then spun for 10 min to remove precipitates and $A_{520\,nm}$ was measured. AHS activity is expressed as the amount (μmol) of acetolactate produced per min per mg of protein.

[c] Final concentration of valine was 0.1 mM, % indicates AHS activity inhibition by valine compared to the control.

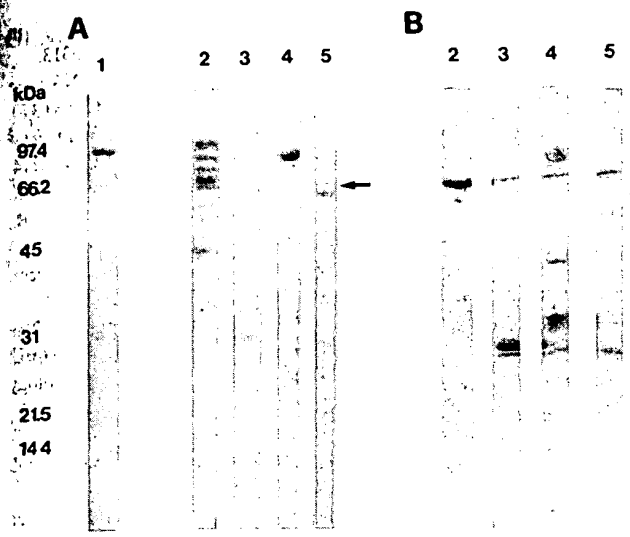


Fig. 3. SDS-PAGE (**A**) and immunoblot (**B**) of *Sa* re-AHS. The separate proteins were stained with Coomassie brilliant blue; the proteins were transferred to nitrocellulose and probed with goat anti-GST Ab. Lanes: 1, low molecular weight markers; 2, *Ec* PS1283 sonicate; 3, GST; 4, GST-AHS fusion protein; 5, GST-AHS fusion protein cleaved with thrombin (the position of the cleaved fusion protein is indicated by an arrow). **Methods:** Recombinant plasmids containing the *Sa ilv* gene sequence under the control of the *tac* promoter were constructed using pGEX-4T-1 (Smith and Johnson, 1988) to create an in-frame fusion to GST. To synthesize this GST fusion protein, a 2.4-kb sequence of *ilv* gene in λEMBL-3 re-phage was amplified by PCR with the synthetic oligos 5′-CGGGATCCATGACCGAGCAGGCCACC and 5′-CGGAATTCCTTACGCGGATCGGTCCAG. *Bam*HI and *Eco*RI sites were generated at the 5′ ends of the primers to allow subsequent ligation with the *Bam*HI + *Eco*RI-treated pGEX-4T-1 vector. PCR was performed with a 40-μl mixture of 10 mM Tris·HCl pH 8.3/50 mM KCl/1.5 mM $MgCl_2$/200 μmol of each dNTP/100 pmol of each primer/10 ng of DNA from λEMBL-3 re-phage (denatured previously at 94°C for 2 min), 2.5 u of Ampli*Taq* polymerase (Perkin-Elmer Cetus). The temperature profile of the first five PCR cycles was 1 min at 94°C, 1 min at 52°C and 3 min at 72°C. After these initial cycles, the samples were subjected to another 25 cycles of 1 min at 94°C, 1 min at 65°C and 3 min at 72°C followed by a further incubation at 72°C for 5 min. The amplified product (2.4 kb), ligated to pGEX-4T-1 vector, was used to electroporate *Ec* JM105 cells. The resulting plasmid was designated pFP1 and the correct reading frame of the chimeric gene was verified by sequencing with 5′ and 3′ pGEX sequencing primers. Complementation of the AHS-deficient strain *Ec* PS1283 (Haughn et al., 1985) with the plasmid pFP1, was tested by transforming this plasmid into PS1283 and plating on minimal medium (Davis and Mingioli, 1950) supplemented with leucine, thymine, tryptophan and Ap. Complementation was observed by the ability of transformants to grow in the absence of valine and isoleucine. *Ec* PS1283 cells harboring the re-plasmid were grown in minimal medium containing 100 μg Ap/ml and induced or not with 0.05–0.1 mM IPTG, and the 92-kDa GST-AHS fusion protein was purified and cleaved with thrombin according to the instructions of the Bulk GST Purification Module (Pharmacia). The fusion protein, before and after thrombin cleavage, was fractionated on by denaturating 0.1% SDS-10% PAGE (Sambrook et al., 1989). Specific detection of the chimeric protein was achieved by Western immunoblot analysis with goat anti-GST Ab and anti-goat IgG peroxidase conjugate.

fusion protein with thrombin resulted in the release of *Sa* re-AHS protein with a molecular mass of 66 kDa (Fig. 3A, lane 5). The GST and the 92-kDa fusion protein reacted strongly with goat anti-GST Ab in conjunction with an anti-goat IgG peroxidase conjugate (Fig. 3B, lanes 3 and 4). A sample of *Ec* PS1283 sonicate, made from a culture which does not contain a pGEX plasmid, was run as a control (Fig. 3A,B, lane 2).

(c) Conclusions

(*1*) In *Sa*, the *ilvB*, *N* and *C* genes are clustered.

(*2*) The AHS large and small subunits are most similar to the homologues from *Caulobacter crescentus* and *Cg*, and IR is most similar to that of *Cg*.

(*3*) The genes encoding AHS from *Sa*, were expressed in *Ec* and could complement an *ilv* mutation.

(*4*) AHS activity was weakly feedback-inhibited by valine.

ACKNOWLEDGEMENTS

We thank Dr. D.A. Hopwood for critical reading of the manuscript. We are grateful also to Dr. Z. Vanek for providing *Sa* CU18 strain and for helpful advice. This work was supported by grants from M.U.R.S.T. 40% and 60%.

REFERENCES

Aguilar, O.M. and Grasso, D.H.: The product of the *Rhizobium meliloti ilvC* gene is required for isoleucine and valine synthesis and nodulation of alfalfa. J. Bacteriol. 173 (1991) 7756–7764.

Chater, K.F.: Genetics of differentiation in *Streptomyces*. Annu. Rev. Microbiol. 47 (1993) 685–713.

Cimburkova, E., Zima, J., Novak, J. and Vanek, Z.: Nitrogen regulation of avermectins biosynthesis in *Streptomyces avermitilis* in a chemically defined medium. J. Basic Microbiol. 38 (1988) 491–499.

Cordes, C., Möckel, B., Eggeling, L. and Sahm, H.: Cloning, organization and functional analysis of *ilvA*, *ilvB* and *ilvC* genes from *Corynebacterium glutamicum*. Gene 112 (1992) 113–116.

Davis, B.D. and Mingioli, E.S.: Mutants of *Escherichia coli* requiring methionine or vitamin B12. J. Bacteriol. 60 (1950) 17–28.

Fink, P.S.: Biosynthesis of the branched-chain amino acids. In: Sonenshein, A.L., Hoch, J.A. and Losick, R. (Eds.), *Bacillus subtilis* and Other Gram-positive Bacteria: Biochemistry, Physiology and Molecular Genetics. American Society for Microbiology, Washington, DC, 1993, pp. 307–317.

Godon, J.J., Chopin, M.C. and Ehrlich, S.D.: Branched-chain amino acid biosynthesis genes in *Lactococcus lactis* subsp. *lactis*. J. Bacteriol. 174 (1992) 6580–6589.

Hafner, E.W., Holley, B.W., Holdom, K.S., Leese, S.E., Wax, R.G., Beck, D. and McArthur, H.A.I.: Branched-chain fatty acid requirement for avermectin production by a mutant of *Streptomyces avermitilis* lacking branched-chain 2-oxoacid dehydrogenase activity. J. Antibiot. 43 (1991) 349–356.

Haughn, G.W., Squires, C.H., De Felice, M., Lago, C.T. and Calvo, J.M.: Unusual organization of the *ilvIH* promoter of *Escherichia coli*. J. Bacteriol. 163 (1985) 186–198.

Keilhauer, C., Eggeling, L. and Sahm, H.: Isoleucine synthesis in *Corynebacterium glutamicum*: molecular analysis of the *ilvB-ilvN-ilvC* operon. J. Bacteriol. 175 (1993) 5595–5603.

Lawther, R.P., Wek, R.C., Lopes, J.M., Pereira, R., Taillon, B.E. and Hatfield, G.W.: The complete nucleotide sequence of the *ilvGMEDA* operon of *Escherichia coli* K-12. Nucleic Acids Res. 15 (1987) 2137–2155.

Milano, A., De Rossi, E., Zanaria, E., Barbierato, L., Ciferri, O. and Riccardi, G.: Molecular characterization of the genes encoding acetohydroxy acid synthase in the cyanobacterium *Spirulina platensis*. J. Gen. Microbiol. 138 (1992) 1399–1408.

Newman, T., Friden, P., Sutton, A. and Freundlich, M.: Cloning and expression of the *ilvB* gene of *Escherichia coli* K-12. Mol. Gen. Genet. 186 (1982) 378–384.

Pospisil, S., Peterkova, M., Krumphanzl, V. and Vanek, Z.: Regulatory mutants of *Streptomyces cinnamonensis* producing monensin A. FEMS Microbiol. Lett. 24 (1984) 209–213.

Sambrook, J., Fritsch, E.F. and Maniatis, T.: Molecular Cloning. A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

Sanger, F., Nicklen, S. and Coulson, A.R.: DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74 (1977) 5463–5467.

Smith, D. and Johnson, K.: Single step purification of polypeptides in *Escherichia coli* as fusions with glutathione *S*-transferase. Gene 67 (1988) 31–40.

Squires, C.H., De Felice, M., Devereux, J. and Calvo, J.M.: Molecular structure of *ilvIH* and its evolutionary relationship to *ilvG* in *Escherichia coli* K-12. Nucleic Acids Res. 11 (1983) 5299–5313.

Suzuki, Y. and Yamada, T.: The nucleotide sequence of 16S rRNA gene from *Streptomyces lividans* TK21. Nucleic Acids Res. 16 (1988) 370.

Tarleton, J.C., Malakooti, J. and Ely, B.: Regulation of *Caulobacter crescentus ilvBN* gene expression. J. Bacteriol. 176 (1994) 3765–3774.

Umbarger, H.E.: Biosynthesis of the branched-chain amino acids. In: Neidhardt, F.C., Ingraham, J.L., Low, K.B., Magasanik, B., Schaechter, M. and Umbarger, H.E. (Eds.), *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology. American Society for Microbiology, Washington, DC, 1987, pp. 352–367.

Vandeyar, M.A., Mackey, C.J., Lipsky, R.H. and Zahler, S.A.: The *ilvBC-leu* operon of *Bacillus subtilis*. In: Ganesan, A.T. and Hoch, J.A. (Eds.), *Bacillus* Molecular Genetics and Biotechnology Applications. Academic Press, New York, NY, 1986, pp. 295–305.

Wek, R.C. and Hatfield, G.W.: Nucleotide sequence and *in vivo* expression of the *ilvY* and *ilvC* genes in *Escherichia coli* K-12. J. Biol. Chem. 261 (1986) 2441–2450.

Wek, R.C., Hauser, C.A. and Hatfield, G.W.: The nucleotide sequence of the *ilvBN* operon of *Escherichia coli*: sequence homologies of the acetohydroxy acid synthase isozymes. Nucleic Acids Res. 13 (1985) 3995–4010.

Yanofsky, C.: The tryptophan operon. In: Neidhardt, F.C., Ingraham, J.L., Low, K.B., Magasanik, B., Schaechter, M. and Umbarger, H.E. (Eds.), *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology. American Society for Microbiology, Washington, DC, 1987, pp. 1453–1472.

STIC-ILL

From: STIC-Biotech/ChemLib
Sent: Wednesday, August 06, 2003 10:40 AM
To: STIC-ILL
Subject: FW: Non patent literature - Serial No. 09/914,006

Importance: High

-----Original Message-----
From: Fronda, Christian
Sent: Wednesday, August 06, 2003 10:28 AM
To: STIC-Biotech/ChemLib
Subject: Non patent literature - Serial No. 09/914,006
Importance: High

I would like to order the following non patent literature listed below. Thank you.

Christian Fronda
Art Unit 1652
Mailbox CM1 10D01
Office CM1 11E03
(703)305-1252

L14 ANSWER 2 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:961953 HCAPLUS
DOCUMENT NUMBER: 124:77988
TITLE: Cloning, sequencing and expression of the ilvBNC gene cluster from Streptomyces avermitilis
AUTHOR(S): De Rossi, Edda; Leva, Raffaella; Gusberti, Laura; Manachini, Pier Luigi; Riccardi, Giovanna
CORPORATE SOURCE: Department of Genetics and Microbiology, University of Pavia, 27100, Pavia, Italy
SOURCE: Gene (1995), 166(1), 127-32
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

L14 ANSWER 5 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:1848 HCAPLUS
DOCUMENT NUMBER: 120:1848
TITLE: Isoleucine synthesis in Corynebacterium glutamicum: molecular analysis of the ilvB-ilvN-ilvC operon
AUTHOR(S): Keilhauer, Carmen; Eggeling, Lothar; Sahm, Hermann
CORPORATE SOURCE: Inst. Biotechnol., Forschungszent., Juelich, D-52425, Germany
SOURCE: Journal of Bacteriology (1993), 175(17), 5595-603
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

JOURNAL OF BACTERIOLOGY, Sept. 1993, p. 5595–5603
0021-9193/93/175595-09$02.00/0

Vol. 175, No. 17

Isoleucine Synthesis in *Corynebacterium glutamicum*: Molecular Analysis of the *ilvB-ilvN-ilvC* Operon

CARMEN KEILHAUER, LOTHAR EGGELING,* AND HERMANN SAHM
Institut für Biotechnologie, Forschungszentrum, D-52425 Jülich, Germany

Received 25 January 1993/Accepted 14 June 1993

Acetohydroxy acid synthase (AHAS) and isomeroreductase (IR) catalyze subsequent reactions in the flux of metabolites towards isoleucine, valine, leucine, and pantothenate. A 4,705-bp DNA fragment from *Corynebacterium glutamicum* known to code for AHAS and IR was sequenced and analyzed by Northern (RNA blot) analysis. As in other bacteria, the AHAS of this gram-positive organism is encoded by two genes, *ilvB* and *ilvN*. Gene disruption verified that these genes encode the single AHAS activity in *C. glutamicum*. The start of *ilvB* was determined by amino-terminal sequencing of a fusion peptide. By Northern analysis of the *ilvBNC* cluster, three in vivo transcripts of 3.9, 2.3, and 1.1 kb were identified, corresponding to *ilvBNC*, *ilvNC*, and *ilvC* messages, respectively. The *ilvC* transcript (encoding IR) was by far the most abundant one. With a clone from which the *ilvB* upstream regions had been deleted, only the *ilvNC* and *ilvC* transcripts were synthesized, and with a clone from which the *ilvN* upstream regions had been deleted, only the smallest *ilvC* transcript was formed. It is therefore concluded that in the *ilv* operon of *C. glutamicum*, three promoters are active. The amounts of the *ilvBNC* and *ilvNC* transcripts increased in response to the addition of α-ketobutyrate to the growth medium. This was correlated to an increase in specific AHAS activity, whereas IR activity was not increased because of the relatively large amount of the *ilvC* transcript present under all conditions assayed. Therefore, the steady-state level of the *ilvBNC* and *ilvNC* messages contributes significantly to the total activity of the single AHAS. The *ilvC* transcript of this operon, however, is regulated independently and present in a large excess, which is in accord with the constant IR activities determined.

Within the highly branched pathway of isoleucine synthesis, acetohydroxy acid synthase (AHAS) is unique because it naturally uses different substrates, allowing the ultimate synthesis of isoleucine (Ile), valine (Val), leucine (Leu), and pantothenate in parallel reactions. Consequently, AHAS activity is complexly regulated at various levels. Up to five isoenzymes exist in enterobacteria, of which three have been extensively studied. AHAS I (*ilvBN*) is feedback sensitive to Val; its expression is controlled by Val and Leu but also by cyclic AMP (8). AHAS II (*ilvGM*) is controlled by Val-, Leu-, and Ile-mediated attenuation. *ilvGM* is embedded in the *ilvGMEDA* operon, which, together with the downstream *ilvYC* genes, codes for all enzymes needed for Ile synthesis (28). AHAS III (*ilvIH*) is feedback sensitive to Val, and its expression is controlled by interaction of the Leu-Lrp protein complex with regulatory regions (32). In addition to these mechanisms for regulating total AHAS activity, each enzyme has a different specificity towards its two possible substrate mixtures, again influencing the total flux through this one reaction (1).

In contrast to this plurality of sophisticated mechanisms for enterobacteria, only limited information is available for gram-positive organisms, mostly focusing on gene structure. Thus, the sequence of the clustered *leu-ilv* genes of *Lactococcus lactis* has recently been established (9). A similar arrangement of *ilv* and *leu* genes is also known for *Bacillus subtilis* but in the inverse order, *ilv-leu* (14). In the latter organism, transcription attenuation is involved in control of the *ilv-leu* operon (10). The other gram-positive organism for which information on AHAS control is available is *Corynebacterium glutamicum*. Physiological studies showed that AHAS activity in *C. glutamicum* is inhibited by valine and that addition of α-ketobutyrate (which is one AHAS substrate) to cultures results in an up to 20-fold increase in specific AHAS activity (7). AHAS, together with isomeroreductase (IR), catalyzing the subsequent reaction in metabolite flow, resides in *C. glutamicum* on a 7-kb DNA fragment (4), suggesting proximity of the corresponding genes, as in *B. subtilis* and *L. lactis*. However, evidence obtained from functional studies with subcloned fragments points to independent expression of AHAS and IR. To analyze the transcriptional control of this *ilv* locus of *C. glutamicum*, we here present its structure together with in vivo transcript formation and response to α-ketobutyrate addition to cultures.

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. All strains and plasmids used are listed in Table 1. *Escherichia coli* was grown on LB medium (19). *C. glutamicum* was grown on the complex medium CGIII (15) or TY (for RNA isolation) or on the minimal medium CGXII, consisting of (per liter) 20 g of $(NH_4)_2SO_4$, 5 g of urea, 1 g of KH_2PO_4, 1 g of K_2HPO_4, 0.25 g of $MgSO_4 \cdot 7H_2O$, 42 g of 3-morpholinopropanesulfonic acid, 10 mg of $CaCl_2$, 10 mg of $FeSO_4 \cdot 7H_2O$, 10 mg of $MnSO_4 \cdot H_2O$, 1 mg of $ZnSO_4 \cdot 7H_2O$, 0.2 mg of $CuSO_4$, 0.02 mg of $NiCl_2 \cdot 6H_2O$, 0.2 mg of biotin (pH 7), 40 g of glucose, and 0.03 mg of protocatechuic acid. When appropriate, kanamycin (50 μg/ml) or ampicillin (40 μg/ml) was added.

Genetic engineering. DNA was isolated from *C. glutamicum* by a modified alkaline extraction procedure with lysozyme (23). In vitro procedures and the analysis of plasmid and chromosomal DNA were done by standard procedures (19). The enzymes used for this purpose were supplied by Boehringer (Mannheim, Germany), as was the

* Corresponding author.

TABLE 1. Bacterial strains and plasmids used

Strain or plasmid	Relevant characteristics	Source or reference
E. coli		
DH5	F⁻ *supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	11
S17-1	Mobilizing donor strain	26
C. glutamicum		
ATCC 13032	Type strain	American Type Culture Collection
CK21-1	ATCC 13032 *ilvB*::pCK21-1; AHAS⁻	This work
Plasmids		
pUC18	Cloning vector; Ap^r	29
pEM1	Mobilizable cloning vector; Km^r *oriT*^{RP4}	23
pGEM3z	Transcription vector; Ap^r	Promega
pRIT21	IgG-binding domain of *spa* (protein A)	18
pCC2-42	pUC18::*ilvBNC* (7.5-kb chromosomal fragment)	4
pKK5	pJC4::*ilvBNC* (5.9-kb chromosomal fragment)	4
pKK5-2	Exonuclease III deletion of pKK5	4
pKK5-5	Exonuclease III deletion of pKK5	4
pCK54	1.6-kb *Pvu*II-*Eco*RI fragment from pCC2-42, blunted and ligated into *Hin*dII site of pUC18	This work
pCK21-1	pEM1::*ilvB* 1,555-bp internal *Xho*I fragment	This work
pCKHy1-1	Fusion of amino-terminal end of *ilvB* in pKK5 with *Eco*RV fragment of *spa* from pRIT21	This work

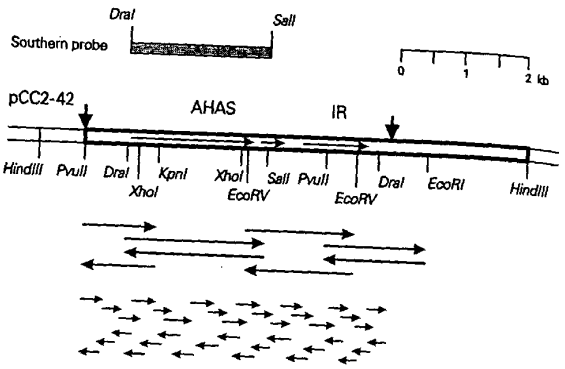


FIG. 1. Overview of Southern analysis and sequencing strategy of the *ilvBNC* cluster (AHAS and IR) of *C. glutamicum*. The *Dra*I-*Sal*I probe was used to verify the integrity of the 7-kb *Pvu*II-*Hin*dIII chromosomal fragment. Also shown are the clones and subclones used for sequencing. The sequence presented in Fig. 2 is bounded by the large vertical arrows.

nonradioactive DNA labeling and detection kit for localizing hybridized DNA and RNA fragments. Plasmids were introduced into *C. glutamicum* via electroporation or by conjugation with *E. coli* S17-1 as the mobilizing donor strain (21). The latter method was used to integrate the nonreplicative plasmid pCK21-1 into the chromosome (24). Plasmid pCK21-1 was constructed by integrating the 1,555-bp *Xho*I fragment of pCC2-42, carrying an internal fragment of *ilvB* (Fig. 1), into the *Sal*I site of the mobilizable vector pEM1 (23).

Protein analysis. To synthesize a fusion protein of the amino-terminal end of the *ilvB* polypeptide with the immunoglobulin G (IgG)-binding domain of the *spa* gene product, a 1,099-bp sequence of *spa* in pRIT21 (18) was amplified by the polymerase chain reaction with the synthetic oligonucleotides 5′-AAGATATCCCTGCTGCCAATGCTGCGC-3′ and 5′-TAAATTCATGCCGGAGAGGG-3′. An *Eco*RV site was thus generated at the 5′ end of *spa* to allow subsequent ligation with the 9.0-kb *Eco*RV fragment of pKK5 (4), providing the amino-terminal end of *ilvB*. The resulting plasmid was designated pCKHy1-1, and the correct reading frame of the chimeric gene was verified by sequencing with synthetic primers.

The wild type of *C. glutamicum* was transformed with pCKHy1-1. The resulting strain was grown to the exponential growth phase on CGXII, harvested by centrifugation, and incubated for 10 min at 95°C to inactivate proteases. After sonification, the synthesized fusion protein was isolated from the cell extract by affinity chromatography with Fast-Flow-IgG-Sepharose (Pharmacia, Piscataway, N.J.). Specific detection of the chimeric protein was achieved by Western immunoblot analysis with rabbit IgG and horseradish peroxidase-linked anti-rabbit IgG antibody. The amino acid sequence of blotted protein was determined by automatic Edman degradation with the Applied Biosystems 477A protein sequencer.

DNA sequencing and analysis. Subclones were made in pUC18 in both orientations, and deletion clones were prepared by exonuclease III treatment (Promega, Madison, Wis.). DNA sequence determination was performed by the dideoxy chain termination method (20) with Klenow enzyme from Boehringer. Sequence data were compiled and analyzed with the HUSAR program package, release 2.0 (EMBL, Heidelberg, Germany). Multiple alignments were carried out with the algorithm of Myers and Miller (17).

RNA isolation and Northern (RNA blot) analysis. RNA analysis was done by the method of Börmann et al. (2) with several modifications. *C. glutamicum* was grown on complex medium TY or minimal medium to the early exponential growth phase. The cultures were chilled on ice for 10 min, harvested at 4°C by centrifugation, and washed twice with 10 ml of ice-cold 50 mM Tris–50 mM NaCl, pH 6.6. The resulting pellet was resuspended in 5 ml of 10 mM sodium acetate–1% sodium dodecyl sulfate (SDS) at pH 5 (65°C), and 5 ml of water-saturated phenol at pH 5.5 (65°C) was added. After incubation for 10 min at 65°C, the aqueous phase was separated by centrifugation, and phenol extraction was repeated two further times. RNA was precipitated with absolute ethanol (−20°C), washed with 70% ethanol, dried, and resuspended in 50 μl of diethylpyrocarbonate-treated water containing 80 U of RNasin (Promega). Quantification by absorbance readings at 260 nm yielded about 300 μg of RNA per 50-ml culture.

For Northern hybridization, about 20 μg of RNA was treated with 1 U of DNase RQ for 10 min at 37°C. Subsequently, 10 μl of loading dye (19) was added, and the sample was heated for 5 min at 95°C and loaded on an agarose gel containing 17% formaldehyde. The separated RNA was transferred to a nylon membrane, and hybridization was performed with digoxygenin-labeled RNA probes.

Four antisense mRNA probes were prepared (see Fig. 3).

As the *ilvB* probe, the 972-bp *Nco*I fragment was cloned into the *Sma*I site of the transcription vector pGEM3z (Promega). After linearization with *Hin*dIII, digoxygenin-labeled antisense mRNA was synthesized in vitro by using T7 or SP6 polymerase and the RNA labeling kit from Boehringer. Likewise, the *ilvN*-specific probe was generated from the 541-bp *Aat*II-*Spe*I fragment, and the *ilvC*-specific probe was generated from the 804-bp *Nco*I-*Cla*I fragment. The fourth probe was prepared from a 1-kb fragment 20 nucleotides downstream of the presumed terminator of the operon. The fragment was isolated as an *Sno*I-*Bam*HI fragment from plasmid pCK54. Hybridization and detection were performed with the nucleic acid detection kit from Boehringer.

Nucleotide sequence accession number. The GenBank accession number of the nucleotide sequence shown in Fig. 2 is L09232.

RESULTS

Primary structure of the *ilv* operon. An overview of the gene arrangement and the sequencing strategy for the cluster are shown in Fig. 1. Within the sequence determined, comprising 4,705 bp, a large coding region is predicted with the stop codon at nucleotide (nt) 2627 and starting either from nt 824, with ATG as the initiation codon, or at nt 749, with the rarer codon GTG (Fig. 2). In order to determine the translational start actually used in vivo, plasmid pCKHyl-1 was constructed, carrying an amino-terminal fusion at nt 1165 with the coding region for the IgG-binding domains of protein A of *Staphylococcus aureus* (27). The plasmid was introduced into *C. glutamicum*, the fusion protein was isolated by a one-step procedure, and its amino acid sequence was determined. The sequence Asn-Val-Ala-Ala-Ser-Gln-Gln-Pro- was obtained, which is in perfect agreement with GTG (nt 749) as the start codon but with the initiator amino acid methionine removed. The second open reading frame, separated by only 13 nt from the first, starts at nt 2643 and ends at nt 3069. In front of this second putative coding region is an appropriate ribosome-binding site, 5′-AAGGAG-3′, which overlaps the stop codon of the first coding region. Since both of the assigned coding regions are located in those parts of DNA fragments functionally identified as resulting in high AHAS activity (4), the genes were named *ilvB* and *ilvN*, in accordance with the *E. coli* and *B. subtilis* nomenclature. The deduced gene products have molecular masses of 66,813 and 15,417 Da, respectively.

The sequence determined was used to apply gene-directed mutation and to disrupt *ilvB* in the chromosome of wild-type *C. glutamicum*. The resulting strain, CK21-1, was devoid of AHAS activity (not shown) and required all three branched-chain amino acids for growth on minimal medium, with somewhat improved growth when pantothenate was also added to the medium. This is proof that, in contrast to the members of the family *Enterobacteriacae* (28), no isoenzymes are present in *C. glutamicum*, but only one single AHAS activity.

Downstream (269 nt) of *ilvN* is the third open reading frame. It corresponds to that DNA region which resulted in high plasmid-encoded IR activity (4) and was therefore designated *ilvC*. It extends from the ATG at nt 3342 (with the ribosome-binding site [5′-GAAAGGCG-3′]) to the TAA at nt 4355. The deduced *ilvC* gene product therefore consists of 338 amino acids, with a molecular mass of 36,159 Da. *ilvC* is followed by a rho-independent terminator-like structure at nt 4394 to 4428, with a ΔG^0 of −23 kcal/mol.

Transcripts of the *ilv* operon. Northern hybridization was used to determine the chromosomally derived mRNA species encoded by the *ilvBNC* operon. As probes, three antisense mRNAs specific to *ilvB*, *ilvN*, and *ilvC* messages were made (Fig. 3). In addition, a fourth probe from the sequence directly downstream of the predicted terminator was synthesized. The *ilvB* gene-specific probe hybridized to a 3.9-kb transcript (Fig. 4, lane 1), which is the size predicted for the full-length *ilvBNC* operon transcript. The *ilvN* and *ilvC* gene-specific probes also hybridized to this transcript (Fig. 4, lanes 2 and 3). Since the *ilvBNC* genes span 3.6 kb of DNA, the 3.9-kb transcript was positioned as shown in Fig. 3, additionally assuming that its 3′ end is located at the predicted terminator downstream of *ilvC*. This is appropriate, since no hybridization was found with the fourth antisense mRNA (Fig. 4, lane 7), which would probe for mRNA directly downstream of the terminator. (Still more evidence comes from deletion clone pKK5-5, described further below.) A shorter transcript of 2.3 kb was detected with either the *ilvN* or *ilvC* gene-specific probe (Fig. 4, lanes 2 and 3). This unexpected transcript is of sufficient length to code for the entire *ilvN* and *ilvC* products and was positioned accordingly, assuming the same termination site as for the 3.9-kb transcript. The third transcript of the cluster detected was 1.1 kb long and hybridized to the *ilvC* gene-specific probe only (Fig. 4, lane 3). The size of this transcript is only slightly larger than that of *ilvC* (which is 1,016 kb) and is once again in very good agreement with termination at the proposed terminator.

We also analyzed the origin of the transcripts, since degradation products (either natural or artificial) are frequently observed during RNA analysis. For this purpose, we took advantage of two subclones of the operon, pKK5-2 and pKK5-5, with *ilvB* or *ilvN* 5′ upstream regions, respectively, removed (Fig. 3). Plasmid pKK5 (with the entire operon) yielded the expected three transcripts (Fig. 4, lane 4), whereas pKK5-2, with the 5′ upstream region of *ilvB* removed (Fig. 3), abolished synthesis of the *ilvBNC* transcript (Fig. 4, lane 5). However, it did not affect synthesis of the two smaller transcripts. This is proof that the *ilvNC* transcript is due to separate transcription initiation and that it does not arise from the large transcript by posttranscriptional events. Moreover, due to the deletion in pKK5-2, the region of the transcription start of the *ilvBNC* message can also be restricted. It is probably located upstream of the *Nar*I site. Plasmid pKK5-5 was used to investigate the origin of the most abundant 1.1-kb transcript. This plasmid still yielded the smallest transcript but not the two larger ones (Fig. 4, lane 6), confirming that its own promoter drives *ilvC* expression, as already suggested from functional studies (4).

In *C. glutamicum*, the AHAS enzyme level is increased by α-ketobutyrate addition because of the imbalance of the two possible substrates of the enzyme (7). We assayed whether this effect is related to the steady-state levels of the operon-derived transcripts. For this purpose, cells were grown on minimal medium with and without α-ketobutyrate, and transcripts and enzyme activities were determined. By comparing lanes 1 and 2 in Fig. 5, it is apparent that the intensity of the hybridization signal of the 3.9-kb transcript was strongly increased in response to α-ketobutyrate addition. This also holds for the 2.3-kb transcript, since lane 2 received only about one-third as much RNA because otherwise dark signals overlapped on the filter. In parallel, the specific AHAS activity was increased about sevenfold (Fig. 5). Therefore, α-ketobutyrate addition results in increased steady-state levels of the *ilvBNC* and *ilvNC* messages, with concomitant increased translation. The IR specific activity

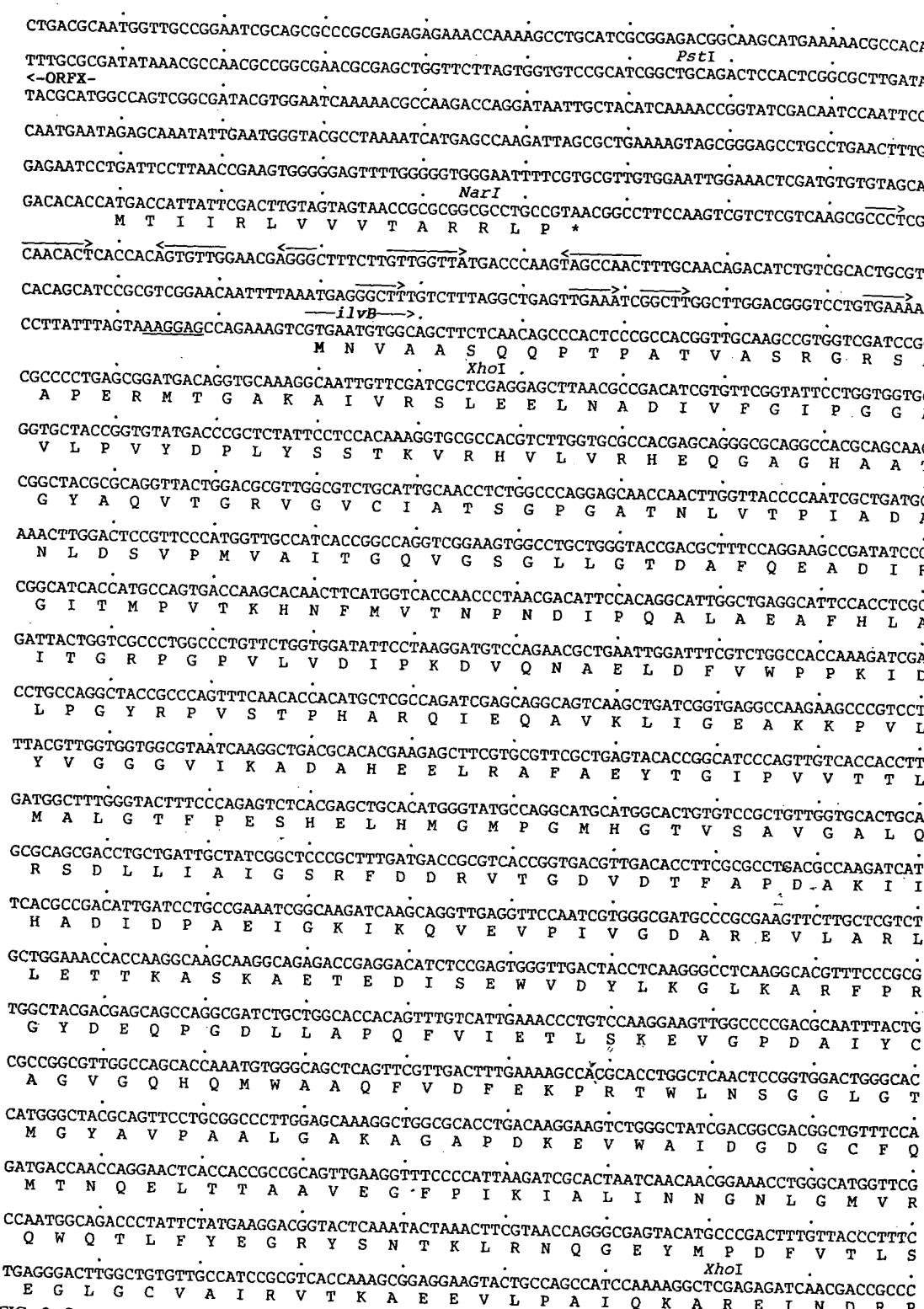


FIG. 2. Sequence of the *ilvBNC* cluster, with deduced polypeptide sequences and selected restriction sites. In front of *ilvB*, a possible leader peptide is given. The three genes of the cluster and the start of an unknown open reading frame (ORFX) are indicated. Stops are indicated by asterisks, the putative Shine-Dalgarno sequences are underlined, and inverted repeats are indicated by arrows.

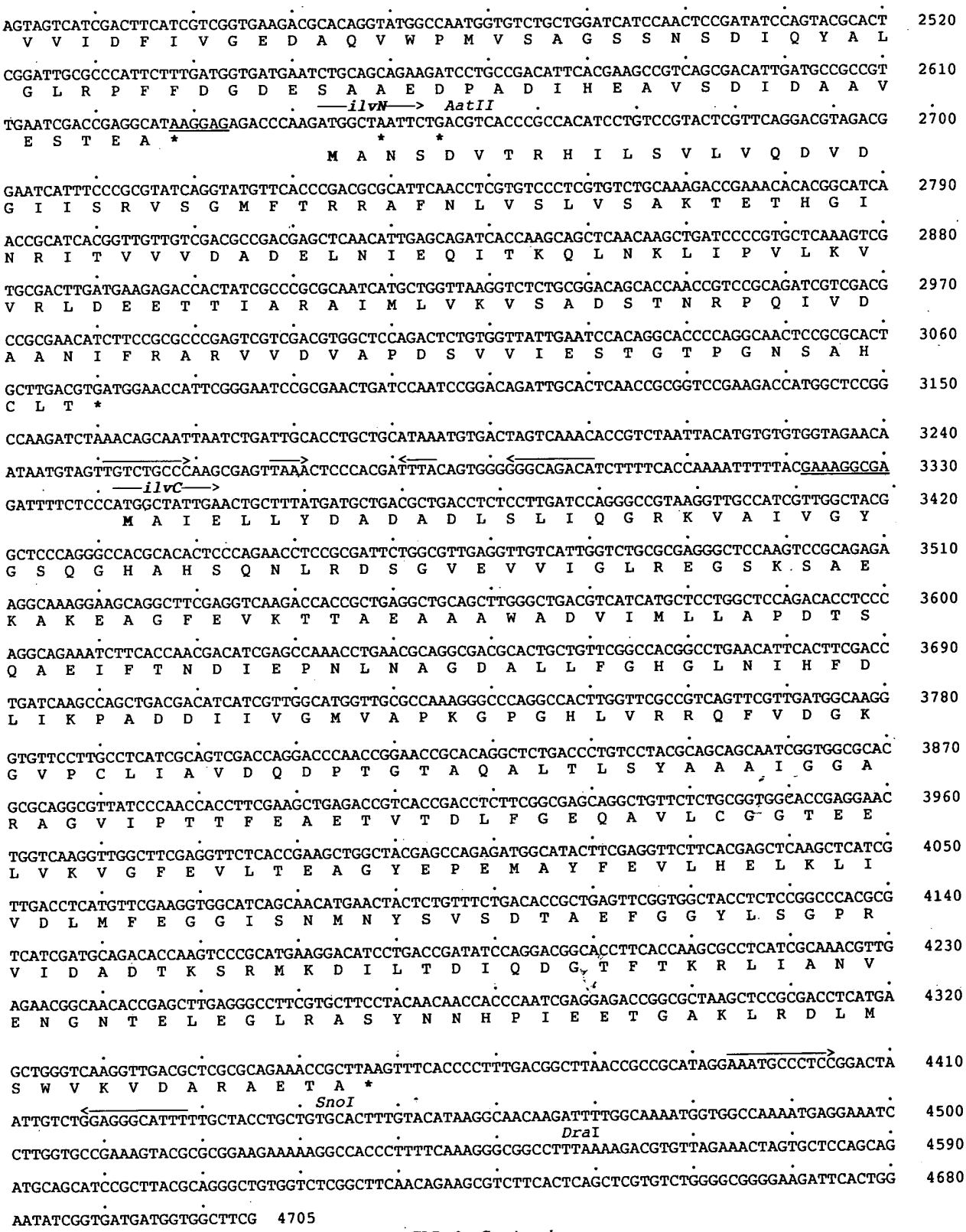


FIG. 2—*Continued.*

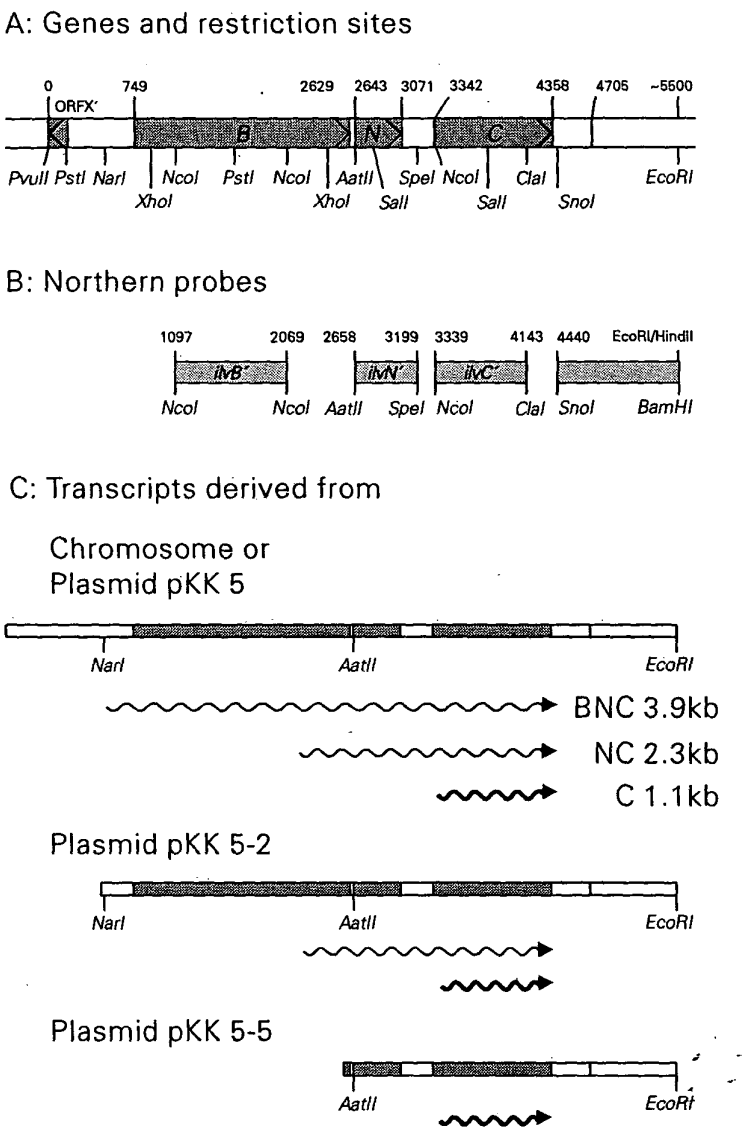


FIG. 3. (A) Locations of the coding regions of the *ilvBNC* cluster and selected restriction enzyme sites. (B) Probes used in Northern blots, which were used as antisense mRNA. (C) In vivo transcripts detected in wild-type *C. glutamicum* and transcripts from plasmids with various 5′ upstream regions of the operon removed. The relative abundances of the three mRNAs mapped by Northern blotting (wavy lines) are indicated by the thickness of the line.

was not increased by α-ketobutyrate addition, reflecting the facts that the *ilvC* transcript is by far the most abundant one of the operon and that the level of this transcript is independent of the presence of α-ketobutyrate. In complex medium, the effect of α-ketobutyrate on AHAS activity was always less pronounced (not shown). Nevertheless, at least a significant effect on the level of the 2.3-kb transcript can be seen (Fig. 5, lane 4). Also, the specific activity of the AHAS was increased, but not that of IR.

DISCUSSION

The established nucleotide sequence of the *ilv* operon of *C. glutamicum* shows that the single AHAS of this organism is encoded by two genes (*ilvB* and *ilvN*), as found for all other bacterial biosynthetic AHAS enzymes (28). It is also apparent on the protein level that AHAS from *C. glutamicum* is remarkably homologous to the group of the biosynthetic AHAS enzymes (also to those of plants with only one subunit), which constitute a structurally related group with pyruvate decarboxylase and pyruvate oxidase (3). In contrast to this group of enzymes, the IR proteins are apparently less uniform in structure. It can be seen from homology comparisons (not shown) that in the *E. coli* (and spinach) polypeptides (6, 13), a stretch of about 135 amino acid residues is inserted which has no counterpart in the *C. glutamicum* IR polypeptide. A specific function could not be attributed either to this additional sequence of amino acids in the *E. coli* polypeptide or to any other part of the IR. We here propose specific domains of the *C. glutamicum* IR polypeptide.

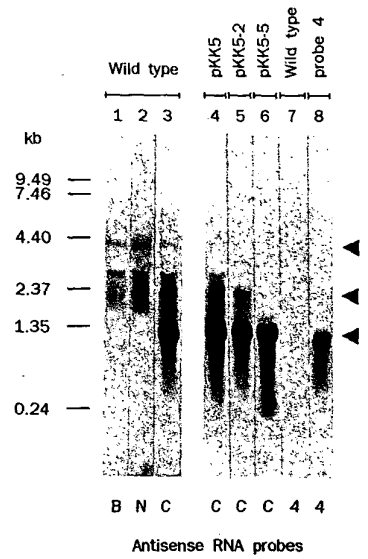


FIG. 4. Northern analysis of the *ilv* operon of *C. glutamicum* ATCC 13032. The *ilvB* probe (lane 1) hybridizes to the 3.9-kb message of the chromosome. The *ilvN* probe (lane 2) hybridizes to the 3.9-kb and 2.3-kb messages. The *ilvC* probe (lane 3) hybridizes to the 3.9-kb, 2.3-kb, and 1.1-kb messages. Recombinants containing plasmids with the entire operon (pKK5, lane 4) or 5′ deletions of the operon (lanes 5 and 6) yielded all three transcripts, the two smaller transcripts, and the smallest transcript, respectively. About 20 μg of RNA was applied to lanes 1 to 3 and 7, and 4 μg was applied to lanes 4 to 6. In lane 7, the terminator downstream probe was used, which did not hybridize to mRNA, although the probe itself (lane 8) was labeled comparably to the operon-derived probes used in lanes 1 to 6. At the left, the positions of RNA standards (GIBCO/BRL, Eggenstein, Germany) are given. Arrowheads indicate the locations of the three transcripts of the cluster.

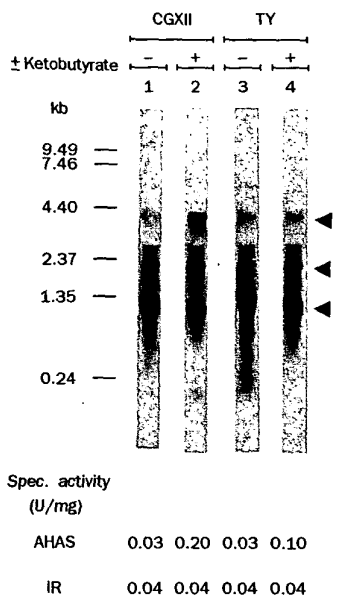


FIG. 5. Northern analysis of the *ilv* operon in response to α-ketobutyrate addition. RNA was extracted from wild-type *C. glutamicum* grown on minimal medium (CGXII) or complex medium (TY) with and without α-ketobutyrate, as indicated. The three different transcripts were monitored with the *ilvC* probe. About 20 μg of RNA was applied to lanes 1, 3, and 4, whereas lane 2 received only 7 μg. Extract was prepared from the same cells for AHAS (*ilvBN*) and IR (*ilvC*) activity determinations.

Within the *C. glutamicum* IR, an almost perfect fit to the ADP fingerprint of NAD-binding domains (31) can be identified at residues 20 to 51. Therefore, this highly conserved sequence is predicted to constitute the β1-αA-β2 structure of the NAD-binding domain, where the glycines at positions 25, 27, and 30 (Fig. 6) mark the tight turn at the end of the first β-strand and the beginning of the succeeding α-helix. The NAD-binding domain in dehydrogenases is about 140 amino acids long and is frequently located at the amino terminus, as is the case for the *C. glutamicum* IR protein. Figure 6 shows a comparison of the IR peptides from *C. glutamicum*, *Lactococcus lactis*, *B. subtilis*, and *Rhizobium meliloti*. A block of high homology (about 40% identical amino acids) is located at residues 1 to 191 of the *C. glutamicum* sequence, the majority of which would therefore represent the NAD-binding domain. An extremely highly conserved block follows the designated NAD-binding domain. It is most conspicuous that within its first 50 amino acid residues, negatively charged and aromatic amino acids are greatly overrepresented. Conserved cysteine and histidine residues are also located in this region. This region is therefore predicted to represent the microenvironment required for the complex two-step reaction of intramolecular alkyl transfer and reduction to the diol of the IR substrate. The F-E-X-L-X-E motif, which occurs twice (starting at positions 212 and 227), is probably directly involved in alkyl migration, since various acid-base mechanisms for its catalysis have been discussed recently (6, 16).

Northern analysis gives the actual sizes and distributions of the *ilvBNC* operon-derived mRNA species. In *C. glutamicum*, we found three transcripts encoded by the *ilvBNC* operon, which are the *ilvBNC*, *ilvNC*, and *ilvC* transcripts. The most abundant *ilvC* transcript was expected from the physiological and genetic investigations, since noncoordinate IR and AHAS expression was found (22), and IR oversynthesis with cloned fragments was unaffected by *ilvB* deletions, as in pKK5-5 (4). Even if the *ilvBNC* and *ilvNC* transcripts are present at higher levels after α-ketobutyrate addition, this is apparently of no significance for the overall IR activity, since because of their relatively small quantity, these large transcripts can make only a marginal contribution to IR synthesis.

The *ilvNC* transcript was unexpected. This is the first case of a transcript also coding for the small subunit of the AHAS. Only recently has a Northern analysis of the *ilvGMEDA* operon of *E. coli* been carried out (12). However, only the full-length operon transcript was found, which codes for both subunits of AHAS. In *C. glutamicum*, however, both the *ilvBNC* and *ilvNC* transcripts might contribute to the synthesis of the small subunit of the AHAS. In this respect, it is interesting that the small subunit is necessary for allosteric control (30), and under in vitro conditions for AHAS III of *E. coli* (*ilvIH*), an excess of the small subunit was shown to be necessary for maximal activity. The additional *ilvNC* transcript in *C. glutamicum* may contribute to the fidelity of the allosteric regulation of AHAS activity.

The three transcripts of the *ilv* operon of *C. glutamicum* are the result of their own transcription initiation, as is evident from plasmids with progressive 5′ deletions. Their localization with respect to the physical map is fairly unambiguous. However, sequence analysis does not allow the

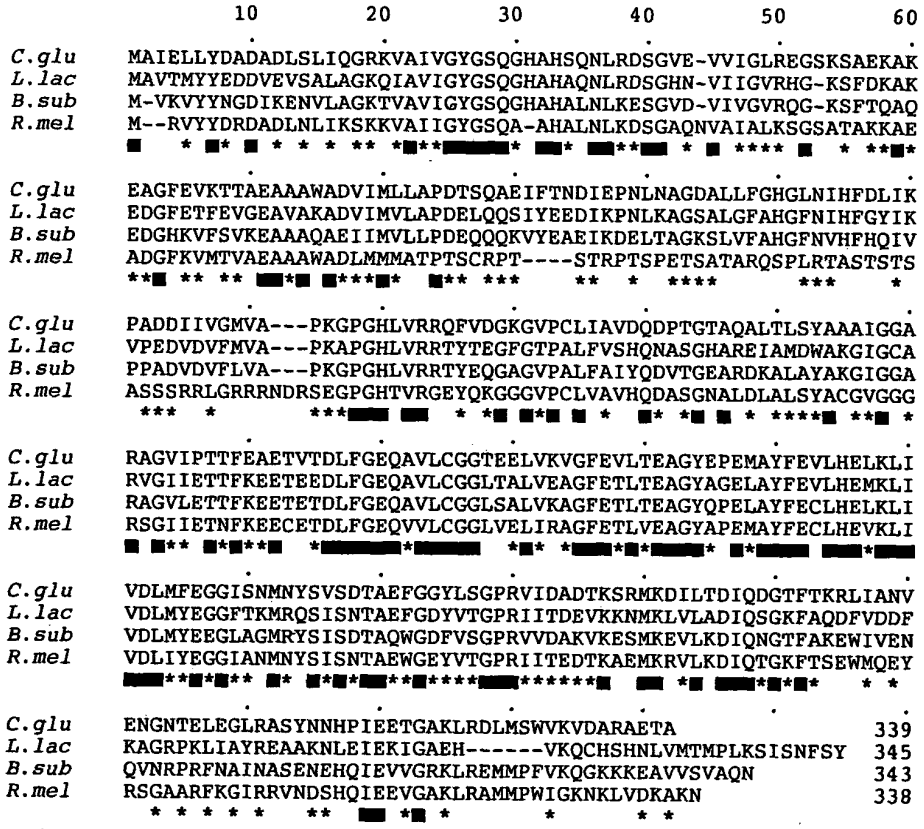


FIG. 6. Alignment of the deduced IR polypeptide sequences from *C. glutamicum* (C.glu), *Lactococcus lactis* (L.lac), *B. subtilis* (B.sub), and *Rhizobium meliloti* (R.mel). Identical (■) and similar (*) amino acids are marked.

corresponding promoters to be assigned, since knowledge about promoter consensus sequences of this gram-positive bacterium is still minimal (25). The length and localization of the *ilvBNC* transcripts, however, permit the conclusion that the *ilvB* promoter is not far upstream of the *Nar*I site. Consistent with this is the fact that in the large untranslated region in front of *ilvB*, several stem-loop structures are present, which are likely involved in controlling *ilvBNC* transcription. Within the *ilv* operon, two prominent stem-loop structures of the corresponding mRNA are present. One is the predicted terminator directly downstream of *ilvC*, which has a ΔG^0 (25°C) of −23 kcal/mol. The second stem-loop is comparably favored (ΔG^0 of −21.4 kcal/mol) but is obviously not a terminator, since no *ilvBN* or *ilvN* transcript was formed. This stem-loop is therefore predicted to stabilize the 5′ end of the *ilvC* transcript.

On comparing the steady-state levels of the various transcripts of the *ilvBNC* operon of *C. glutamicum* and the *ilvGMEDA* operon of *E. coli* (12), it is obvious that the full-length operon transcripts coding for AHAS are present in comparably small amounts. The AHAS key reaction important for the flux to Ile, Val, Leu, and pantothenate is thus more strongly controlled than the downstream gene(s) not only on the biochemical level (28) but also genetically. A close correlation of the full-length operon transcripts with the flux is therefore conceivable. It has been reported that the addition of α-ketobutyrate (7) or hydroxybutyrate (22) to *C. glutamicum* results in a high flux of precursors towards Ile. It is speculated that this is a result of a Val and Leu shortage in the cells (5, 9). It is now known that this α-ketobutyrate effect is related to an increased steady-state level of the *ilvBNC* transcript, and the present investigation will enable the molecular effects of AHAS expression to be studied in detail.

ACKNOWLEDGMENTS

We thank B. Eikmanns for discussion and advice with RNA work, K. Schimz for help in protein isolation, L. Elling for protein sequencing, and M. Uhlén for plasmid pRIT21.

This work is part of a joint project with Degussa AG and is supported by grant 0319256A8 from the Bundesministerium für Forschung und Technologie.

REFERENCES

1. **Barak, Z., D. M. Chipman, and N. Gollop.** 1987. Physiological implications of the specificity of acetohydroxy acid synthase isozymes of enteric bacteria. J. Bacteriol. **169**:3750–3756.
2. **Börmann, E., B. J. Eikmanns, and H. Sahm.** 1992. Molecular analysis of the *Corynebacterium glutamicum gdh* gene encoding glutamate dehydrogenase. Mol. Microbiol. **6**:317–326.
3. **Chang, Y.-Y., and J. E. Cronan, Jr.** 1988. Common ancestry of *Escherichia coli* pyruvate oxidase and the acetohydroxy acid synthases of the branched-chain amino acid biosynthetic pathway. J. Bacteriol. **170**:3937–3945.
4. **Cordes, C., B. Möckel, L. Eggeling, and H. Sahm.** 1992. Cloning, organization and functional analysis of *ilvA*, *ilvB*, and *ilvC* genes from *Corynebacterium glutamicum*. Gene **112**:113–116.
5. **De Felice, M., M. Newman, and T. Levinthal.** 1978. Regulation of synthesis of the acetohydroxy acid synthase I isoenzyme in *Escherichia coli*. Biochim. Biophys. Acta **541**:1–8.
6. **Dumas, R., M. Lebrun, and R. Douce.** 1991. Isolation, characterization and sequence analysis of a full-length cDNA clone

encoding acetohydroxy acid reductoisomerase from spinach chloroplasts. Biochem. J. **267**:469–475.
7. **Eggeling, I., C. Cordes, L. Eggeling, and H. Sahm.** 1987. Regulation of acetohydroxy acid synthase in *Corynebacterium glutamicum* during fermentation of α-ketobutyrate to L-isoleucine. Appl. Microbiol. Biotechnol. **25**:346–351.
8. **Friden, P., P. Tsui, K. Okamoto, and M. Freundlich.** 1984. Interaction of cyclic AMP receptor protein with the *ilvB* biosynthetic operon in *E. coli*. Nucleic Acids Res. **12**:8145–8160.
9. **Godon, J., M. Chopin, and S. D. Ehrlich.** 1992. Branched-chain amino acid biosynthesis genes in *Lactococcus lactis* subsp. *lactis*. J. Bacteriol. **174**:6580–6589.
10. **Grandoni, J. A., S. Zahler, and J. M. Calvo.** 1992. Transcriptional regulation of the *ilv-leu* operon of *Bacillus subtilis*. J. Bacteriol. **174**:3212–3219.
11. **Hanahan, D.** 1985. Techniques for transformation of *E. coli*, p. 109–136. *In* D. M. Glover (ed.), DNA cloning, vol. 1. IRL Press, Oxford.
12. **Huang, F., G. Coppola, and D. H. Calhoun.** 1992. Multiple transcripts encoded by the *ilvGMEDA* gene cluster of *Escherichia coli* K-12. J. Bacteriol. **174**:4871–4877.
13. **Lawther, R. P., R. C. Wek, J. M. Lopes, R. Pereira, B. E. Taillon, and W. Hatfield.** 1987. The complete nucleotide sequence of the *ilvGMEDA* operon of *Escherichia coli* K-12. Nucleic Acids Res. **15**:2127–2155.
14. **Mackey, C. J., R. J. Warburg, H. O. Halvorson, and S. A. Zahler.** 1984. Genetic and physical analysis of the *ilvBC-leu* region in *Bacillus subtilis*. Gene **32**:49–56.
15. **Menkel, E., G. Thierbach, L. Eggeling, and H. Sahm.** 1989. Influence of increased aspartate availability on lysine formation by a recombinant strain of *Corynebacterium glutamicum* and utilization of fumarate. Appl. Environ. Microbiol. **55**:684–688.
16. **Mrachko, G. T., S. K. Chunduru, and K. C. Calvo.** 1992. The pH dependence of the kinetic parameters of ketol acid reductoisomerase indicates a proton shuttle mechanism for alkyl migration. Arch. Biochem. Biophys. **294**:446–453.
17. **Myers, E. W., and W. Miller.** 1988. Optimal alignments in linear space. Cabios **4**:11–17.
18. **Nilsson, B., and L. Abrahamsen.** 1990. Gene expression technology. Methods Enzymol. **185**:144–161.
19. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
20. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
21. **Schäfer, A., J. Kalinowski, R. Simon, A. Seep-Feldhaus, and A. Pühler.** 1990. High-frequency conjugal plasmid transfer from gram-negative *Escherichia coli* to various gram-positive coryneform bacteria. J. Bacteriol. **172**:1663–1666.
22. **Scheer, E., C. Cordes, L. Eggeling, and H. Sahm.** 1987. Regulation of acetohydroxy acid synthase in *Corynebacterium glutamicum* during isoleucine formation from α-hydroxybutyric acid. Arch. Microbiol. **149**:173–174.
23. **Schrumpf, B., A. Schwarzer, J. Kalinowski, A. Pühler, L. Eggeling, and H. Sahm.** 1991. A functionally split pathway for lysine synthesis in *Corynebacterium glutamicum*. J. Bacteriol. **173**:4510–4516.
24. **Schwarzer, A., and A. Pühler.** 1990. Genetic manipulation of the amino acid-producing *Corynebacterium glutamicum* strain ATCC 13032 by gene disruption and gene replacement. Bio/Technology **9**:84–87.
25. **Schwinde, J. W., N. Thum-Schmitz, B. J. Eikmanns, and H. Sahm.** 1993. Transcriptional analysis of the *gap-pgk-tpi-ppc* gene cluster of *Corynebacterium glutamicum*. J. Bacteriol. **175**:3905–3908.
26. **Simon, R., U. Priefer, and A. Pühler.** 1983. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram-negative bacteria. Bio/Technology **1**:784–791.
27. **Uhlén, M., M. Lindberg, B. Guss, B. Nilsson, S. Gatenbeck, and L. Philison.** 1984. Complete nucleotide sequence of the staphylococcal gene encoding protein A. J. Biol. Chem. **259**:1695–1702.
28. **Umbarger, H. E.** 1987. Biosynthesis of the branched-chain amino acids, p. 352–367. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
29. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
30. **Weinstock, O., C. Sella, D. M. Chipman, and Z. Barak.** 1992. Properties of subcloned subunits of bacterial acetohydoxy acid synthases. J. Bacteriol. **174**:5560–5566.
31. **Wierenga, R. K., P. Terpstra, and W. G. J. Hol.** 1986. Prediction of the occurrence of the ADP-binding βαβ-fold in proteins, using an amino acid sequence fingerprint. J. Mol. Biol. **187**:101–107.
32. **Willins, D. A., C. W. Ryan, J. V. Platko, and J. M. Calvo.** 1991. Characterization of Lrp, an *Escherichia coli* regulatory protein that mediates a global response to leucine. J. Biol. Chem. **266**:10768–10774.

=> d ibib ab 1

ilvD

1026 on c1, 4, 6, 7, 8, 9

L10 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1996:342147 HCAPLUS
DOCUMENT NUMBER: 125:4414
TITLE: Cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and its use for manufacturing isoleucine and valine
INVENTOR(S): Inui, Masayuki; Man, Tomoko; Kobayashi, Miki; Yugawa, Hideaki
PATENT ASSIGNEE(S): Mitsubishi Chem Corp, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

ABSTRACT

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 08089249	A2	19960409	JP 1994-234612	19940929

PRIORITY APPLN. INFO.: JP 1994-234612 19940929

AB The gene encoding dihydroxy-acid dehydratase (E.C. 4.2.1.9) is isolated from Brevibacterium flavum strain MJ-233. Expression plasmid pCRY30-DH encoding the enzyme was prepd. and used for the transformation of coryneform bacteria. Brevibacterium flavum strain MJ-233 transformed with the plasmid produced isoleucine 20 mM into the medium as compared to 10 mM by the wild type.

Brevibacterium = Corynebacterium

=> d ia11

L10 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1996:342147 HCAPLUS
DOCUMENT NUMBER: 125:4414
TITLE: Cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and its use for manufacturing isoleucine and valine
INVENTOR(S): Inui, Masayuki; Man, Tomoko; Kobayashi, Miki; Yugawa, Hideaki
PATENT ASSIGNEE(S): Mitsubishi Chem Corp, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
INT. PATENT CLASSIF.:
MAIN: C12N015-09
ADDITIONAL: C12N009-88
INDEX: C12N015-09, C12R001-13; C12N009-88, C12R001-13
CLASSIFICATION: 7-2 (Enzymes)
Section cross-reference(s): 10
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 08089249	A2	19960409	JP 1994-234612	19940929

PRIORITY APPLN. INFO.: JP 1994-234612 19940929
ABSTRACT:
The gene encoding dihydroxy-acid dehydratase (E.C. 4.2.1.9) is isolated from Brevibacterium flavum strain MJ-233. Expression plasmid pCRY30-DH encoding the enzyme was prepd. and used for the transformation of coryneform bacteria. Brevibacterium flavum strain MJ-233 transformed with the plasmid produced isoleucine 20 mM into the medium as compared to 10 mM by the wild type.

SUPPL. TERM: coryneform bacteria dihydroxy acid dehydratase gene; valine isoleucine manuf Brevibacterium
INDEX TERM: Brevibacterium flavum
Deoxyribonucleic acid sequences
Protein sequences
(cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and use for manufg. isoleucine and valine)
INDEX TERM: Gene, microbial
ROLE: MSC (Miscellaneous)
(cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and use for manufg. isoleucine and valine)
INDEX TERM: Plasmid and Episome
(pCRY30-DH; expression of gene for dihydroxy-acid dehydratase of Brevibacterium flavum on)
INDEX TERM: Bacteria
(coryneform, cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and use for manufg. isoleucine and valine)
INDEX TERM: 177474-84-9
ROLE: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence; cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and use for manufg. isoleucine and valine)
INDEX TERM: **72-18-4P**, **Valine**, preparation 73-32-5P, Isoleucine, preparation
ROLE: BPN (Biosynthetic preparation); BIOL (Biological study); **PREP (Preparation)**
(cloning of gene for **dihydroxy-acid dehydratase** of coryneform bacteria and use for manufg. isoleucine and **valine**)
INDEX TERM: 9024-32-2, Dihydroxy-acid dehydratase

ROLE: BUU (Biological use, unclassified); PRP (Properties);
BIOL (Biological study); USES (Uses)
(cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and use for manufg. isoleucine and valine)

INDEX TERM: 177474-83-8
ROLE: BUU (Biological use, unclassified); PRP (Properties);
BIOL (Biological study); USES (Uses)
(nucleotide sequence; cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and use for manufg. isoleucine and valine)

L7 ANSWER 2 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:195478 HCAPLUS
DOCUMENT NUMBER: 130:249283
TITLE: Genetic and physiological analysis of the formation of pantothenate and valine in Corynebacterium glutamicum
AUTHOR(S): Reuter, Uwe
CORPORATE SOURCE: Inst. Biotechnologie, Forschungszentrum Juelich G.m.b.H., Juelich, D-52425, Germany
SOURCE: Berichte des Forschungszentrums Juelich (1998***), Juel-3606, 1-115 pp.
CODEN: FJBEE5; ISSN: 0366-0885
DOCUMENT TYPE: Report
LANGUAGE: German

AB The Gram-pos. bacterium C. glutamicum is used for the prodn. of amino acids, e.g. of L-glutamate and L-Lys. The biosynthetic pathway of pantothenic acid of this organism was elucidated, and the formation of L-Val and D-pantothenic acid to enable a microbiol. prodn. of these compds was increased. The genes panB and panC were cloned, which encode ketopantoate hydroxymethyltransferase and pantothenate synthetase. The 2 enzymes catalyze important steps of the biosynthetic pathway of pantothenate. Sequence anal. revealed that panB comprises 813 bp and panC 837 bp. The genes are organized as an operon. Assays for the enzymes of the pathway were developed. The pantothenate synthetase has a sp. activity of 1 nmol/min-mg protein, ketopantoate hydroxymethyltransferase one of 0.14 nmol/min-mg protein, and the aspartate .alpha.-decarboxylase one of 0.11 nmol/min-mg protein. The quant. anal. of the formation of pantothenic acid revealed that C. glutamicum accumulates 10 .mu.g pantothenic acid/L. A system to isolate mutants with an increased formation of pantothenate, which is based on a deficiency of pantothenic acid induced by .alpha.-ketobutyrate, was established. The application of this method lad to the isolation of a mutant which accumulates 250 .mu.g pantothenate and 1.4 g valine/L. Overexpression of the genes of the valine and isoleucine biosynthetic pathway (ilvBNCD), in combination with the ***deletion of the threonine dehydratase gene ilvA, resulted in the construction of a strain which accumulates 11.3 g valine and 190 mg pantothenate/L. Addnl. overexpression of panBC led to an accumulation of .ltoreq.1 g pantothenic acid/L. Thus, an increase of the formation of pantothenic acid in C. glutamicum by a factor of 105 has been achieved.

REFERENCE COUNT: 163 THERE ARE 163 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

ilvBNCD + delt ilvA

(ABSTRACT)

102 of

c 1-4, 6-12, 14, 16, 17

Fronda, Christian

From: Fronda, Christian
Sent: Wednesday, August 06, 2003 11:17 AM
T : STIC-Biotech/ChemLib
Subject: Non patent literature for Serial NO.09/914,006

I would like the English version of the non patent literature listed below for Serial NO. 09/914,006 if available.

Here is the website that shows the English abstract of the publication that I need Die Zentralbibliothek

Thank you.

Christian Fronda
Art Unit 1652
Mailbox CM1 10D01
Office CM1 11E03
(703)305-1252

L7 ANSWER 2 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:195478 HCAPLUS
DOCUMENT NUMBER: 130:249283
TITLE: Genetic and physiological analysis of the formation of pantothenate and valine in Corynebacterium glutamicum
AUTHOR(S): Reuter, Uwe
CORPORATE SOURCE: Inst. Biotechnologie, Forschungszentrum Juelich G.m.b.H., Juelich, D-52425, Germany
SOURCE: Berichte des Forschungszentrums Juelich (1998***), Juel-3606, 1-115 pp.
CODEN: FJBEE5; ISSN: 0366-0885
DOCUMENT TYPE: Report
LANGUAGE: German

L10 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:195478 HCAPLUS
DOCUMENT NUMBER: 130:249283
TITLE: Genetic and physiological analysis of the formation of pantothenate and valine in Corynebacterium glutamicum
AUTHOR(S): Reuter, Uwe
CORPORATE SOURCE: Inst. Biotechnologie, Forschungszentrum Juelich G.m.b.H., Juelich, D-52425, Germany
SOURCE: Berichte des Forschungszentrums Juelich (1998), Juel-3606, 1-115 pp.
CODEN: FJBEE5; ISSN: 0366-0885
DOCUMENT TYPE: Report
LANGUAGE: German

AB The Gram-pos. bacterium C. glutamicum is used for the prodn. of amino acids, e.g. of L-glutamate and L-Lys. The biosynthetic pathway of pantothenic acid of this organism was elucidated, and the formation of L-Val and D-pantothenic acid to enable a microbiol. prodn. of these compds was increased. The genes panB and panC were cloned, which encode **ketopantoate hydroxymethyltransferase** and **pantothenate synthetase**. The 2 enzymes catalyze important steps of the biosynthetic pathway of pantothenate. Sequence anal. revealed that panB comprises 813 bp and panC 837 bp. The genes are organized as an operon. Assays for the enzymes of the pathway were developed. The **pantothenate synthetase** has a sp. activity of 1 nmol/min-mg protein, **ketopantoate hydroxymethyltransferase** one of 0.14 nmol/min-mg protein, and the aspartate .alpha.-decarboxylase one of 0.11 nmol/min-mg protein. The quant. anal. of the formation of pantothenic acid revealed that C. glutamicum accumulates 10 .mu.g pantothenic acid/L. A system to isolate mutants with an increased formation of pantothenate, which is based on a deficiency of pantothenic acid induced by .alpha.-ketobutyrate, was established. The application of this method lad to the isolation of a mutant which accumulates 250 .mu.g pantothenate and 1.4 g **valine** /L. Overexpression of the genes of the **valine** and isoleucine biosynthetic pathway (ilvBNCD), in combination with the deletion of the threonine dehydratase gene ilvA, resulted in the construction of a strain which accumulates 11.3 g **valine** and 190 mg pantothenate/L. Addnl. overexpression of panBC led to an accumulation of .ltoreq.1 g pantothenic acid/L. Thus, an increase of the formation of pantothenic acid in C. glutamicum by a factor of 105 has been achieved.

L10 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:195478 HCAPLUS
DOCUMENT NUMBER: 130:249283
TITLE: Genetic and physiological analysis of the formation of pantothenate and valine in Corynebacterium glutamicum
AUTHOR(S): Reuter, Uwe
CORPORATE SOURCE: Inst. Biotechnologie, Forschungszentrum Juelich G.m.b.H., Juelich, D-52425, Germany
SOURCE: Berichte des Forschungszentrums Juelich (1998), Juel-3606, 1-115 pp.
CODEN: FJBEE5; ISSN: 0366-0885
DOCUMENT TYPE: Report
LANGUAGE: German

AB The Gram-pos. bacterium C. glutamicum is used for the prodn. of amino acids, e.g. of L-glutamate and L-Lys. The biosynthetic pathway of pantothenic acid of this organism was elucidated, and the formation of L-Val and D-pantothenic acid to enable a microbiol. prodn. of these compds was increased. The genes panB and panC were cloned, which encode **ketopantoate hydroxymethyltransferase** and **pantothenate synthetase**. The 2 enzymes catalyze important steps of the biosynthetic pathway of pantothenate. Sequence anal. revealed that panB comprises 813 bp and panC 837 bp. The genes are organized as an operon. Assays for the enzymes of the pathway were developed. The **pantothenate synthetase** has a sp. activity of 1 nmol/min-mg protein, **ketopantoate hydroxymethyltransferase** one of 0.14 nmol/min-mg protein, and the aspartate .alpha.-decarboxylase one of 0.11 nmol/min-mg protein. The quant. anal. of the formation of pantothenic acid revealed that C. glutamicum accumulates 10 .mu.g pantothenic acid/L. A system to isolate mutants with an increased formation of pantothenate, which is based on a deficiency of pantothenic acid induced by .alpha.-ketobutyrate, was established. The application of this method lad to the isolation of a mutant which accumulates 250 .mu.g pantothenate and 1.4 g **valine** /L. Overexpression of the genes of the **valine** and isoleucine biosynthetic pathway (ilvBNCD), in combination with the deletion of the threonine dehydratase gene ilvA, resulted in the construction of a strain which accumulates 11.3 g **valine** and 190 mg pantothenate/L. Addnl. overexpression of panBC led to an accumulation of .ltoreq.1 g pantothenic acid/L. Thus, an increase of the formation of pantothenic acid in C. glutamicum by a factor of 105 has been achieved.

```
=> s 9024-34-4/rn
L1            1 9024-34-4/RN

=> d

L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2003 ACS on STN
RN   9024-34-4  REGISTRY
CN   Dehydratase, threonine (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   E.C. 4.2.1.16
CN   L-Threonine deaminase
CN   L-Threonine dehydratase
CN   L-Threonine-serine dehydratase
CN   Threonine deaminase
CN   Threonine dehydrase
CN   Threonine dehydratase
MF   Unspecified
CI   MAN
LC   STN Files:   AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA,
       CAPLUS, CASREACT, CIN, EMBASE, TOXCENTER, USPAT2, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
             851 REFERENCES IN FILE CA (1947 TO DATE)
               1 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
             852 REFERENCES IN FILE CAPLUS (1947 TO DATE)
```

i/v A

> d 12

; lvBN

L2 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2003 ACS on STN
RN **9027-45-6** REGISTRY
CN Synthase, acetolactate (9CI) (CA INDEX NAME)
OTHER NAMES:
CN .alpha.-Acetohydroxy acid synthetase
CN .alpha.-Acetohydroxyacid synthase
CN .alpha.-Acetolactate synthase
CN .alpha.-Acetolactate synthetase
CN Acetohydroxy acid synthase
CN Acetohydroxy acid synthetase
CN Acetolactate synthase
CN Acetolactate synthetase
CN Acetolactic synthetase
CN E.C. 4.1.3.18
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA, CAPLUS, CASREACT, CEN, CIN, EMBASE, PROMT, TOXCENTER, USPAT2, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
1168 REFERENCES IN FILE CA (1947 TO DATE)
6 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
1169 REFERENCES IN FILE CAPLUS (1947 TO DATE)

```
=> s 9075-02-9/rn
L9            1 9075-02-9/RN

=> d

L9   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2003 ACS on STN
RN   9075-02-9  REGISTRY
CN   Isomerase, ketol acid reducto- (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   .alpha.-Keto-.beta.-hydroxylacyl reductoisomerase
CN   2-Hydroxy-3-keto acid reductoisomerase
CN   Acetohydroxy acid isomeroreductase
CN   Acetohydroxy acid reductoisomerase
CN   Acetolactate reductoisomerase
CN   Dehydrogenase, dihydroxyisovalerate (isomerizing)
CN   Dihydroxyisovalerate dehydrogenase (isomerizing)
CN   E.C. 1.1.1.86
CN   E.C. 1.1.1.89
CN   Isomeroreductase
CN   Ketol-acid reductoisomerase
CN   Reductoisomerase
DR   37250-22-9, 37250-25-2, 37339-10-9
MF   Unspecified
CI   MAN
LC   STN Files:   ADISNEWS, AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA,
       CAPLUS, CEN, CIN, EMBASE, TOXCENTER, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
             189 REFERENCES IN FILE CA (1947 TO DATE)
               2 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
             189 REFERENCES IN FILE CAPLUS (1947 TO DATE)
```

ilv C

L1 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2003 ACS on STN
RN **9024-32-2** REGISTRY
CN Dehydratase, dihydroxy acid (9CI) (CA INDEX NAME)
OTHER NAMES:
CN .alpha.,.beta.-Dihydroxy acid dehydratase
CN .alpha.,.beta.-Dihydroxyisovalerate dehydratase
CN 2,3-Dihydroxyisovalerate dehydratase
CN Acetohydroxyacid dehydrase
CN Dihydroxy acid dehydrase
CN Dihydroxy acid dehydratase
CN E.C. 4.2.1.9
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, CHEMINFORMRX, EMBASE, TOXCENTER, USPATFULL

ilvD

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
189 REFERENCES IN FILE CA (1947 TO DATE)
189 REFERENCES IN FILE CAPLUS (1947 TO DATE)

```
=> s 56093-17-5/rn
L1            1 56093-17-5/RN

=> d
```

panB

```
L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2003 ACS on STN
RN   56093-17-5  REGISTRY
CN   Hydroxymethyltransferase, 3-methyl-2-oxobutanoate (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   .alpha.-Ketoisovalerate hydroxymethyltransferase
CN   3-Methyl-2-oxobutanoate hydroxymethyltransferase
CN   E.C. 2.1.2.11
CN   Hydroxymethyltransferase, ketopantoate
CN   Ketopantoate hydroxymethyltransferase
CN   Oxopantoate hydroxymethyltransferase
MF   Unspecified
CI   MAN
LC   STN Files:   BIOSIS, BIOTECHNO, CA, CAPLUS, EMBASE, IFICDB, IFIPAT,
       IFIUDB, TOXCENTER, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
              55 REFERENCES IN FILE CA (1947 TO DATE)
               1 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
              55 REFERENCES IN FILE CAPLUS (1947 TO DATE)
```

> s 9023-49-8/rn
L2 1 9023-49-8/RN

=> d

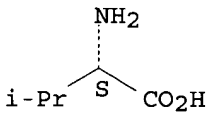
pan C

L2 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2003 ACS on STN
RN **9023-49-8** REGISTRY
CN Synthetase, pantothenate (9CI) (CA INDEX NAME)
OTHER NAMES:
CN E.C. 6.3.2.1
CN Pantoate-activating enzyme
CN Pantoic-activating enzyme
CN Pantothenate synthase
CN Pantothenate synthetase
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, BIOSIS, CA, CAPLUS, TOXCENTER, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
62 REFERENCES IN FILE CA (1947 TO DATE)
62 REFERENCES IN FILE CAPLUS (1947 TO DATE)

L2 ANSWER 2 OF 2 REGISTRY COPYRIGHT 2003 ACS on STN
RN 72-18-4 REGISTRY
CN L-Valine (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Valine, L- (8CI)
OTHER NAMES:
CN (S)-.alpha.-Amino-.beta.-methylbutyric acid
CN (S)-2-Amino-3-methylbutanoic acid
CN (S)-2-Amino-3-methylbutyric acid
CN (S)-Valine
CN 2-Amino-3-methylbutanoic acid
CN Butanoic acid, 2-amino-3-methyl-, (S)-
CN L-(+)-.alpha.-Aminoisovaleric acid
CN L-.alpha.-Amino-.beta.-methylbutyric acid
CN NSC 76038
CN **Valine**
FS STEREOSEARCH
DR 7004-03-7, 16872-32-5
MF C5 H11 N O2
CI COM
LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, AQUIRE, BEILSTEIN*, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA, CANCERLIT, CAOLD, CAPLUS, CASREACT, CBNB, CEN, CHEMCATS, CHEMINFORMRX, CHEMLIST, CIN, CSCHEM, CSNB, DDFU, DETHERM*, DIOGENES, DRUGU, EMBASE, GMELIN*, HODOC*, IFICDB, IFIPAT, IFIUDB, IPA, MEDLINE, MRCK*, MSDS-OHS, NAPRALERT, NIOSHTIC, PIRA, PROMT, RTECS*, SPECINFO, SYNTHLINE, TOXCENTER, TULSA, USAN, USPAT2, USPATFULL
(*File contains numerically searchable property data)
Other Sources: DSL**, EINECS**, TSCA**, WHO
(**Enter CHEMLIST File for up-to-date regulatory information)

Absolute stereochemistry. Rotation (+).



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

23576 REFERENCES IN FILE CA (1947 TO DATE)
662 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
23618 REFERENCES IN FILE CAPLUS (1947 TO DATE)
4 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

=> d his

(FILE 'HOME' ENTERED AT 09:05:06 ON 06 AUG 2003)

FILE 'REGISTRY' ENTERED AT 09:05:13 ON 06 AUG 2003
L1 1 S 9024-32-2/RN
L2 2 S VALINE/CN
L3 1 S 72-18-4/RN

FILE 'HCAPLUS' ENTERED AT 09:07:21 ON 06 AUG 2003

FILE 'REGISTRY' ENTERED AT 09:07:26 ON 06 AUG 2003
SET SMARTSELECT ON
L4 SEL L1 1- CHEM : 8 TERMS
SET SMARTSELECT OFF

FILE 'HCAPLUS' ENTERED AT 09:07:27 ON 06 AUG 2003
L5 210 S L4

FILE 'REGISTRY' ENTERED AT 09:07:35 ON 06 AUG 2003
SET SMARTSELECT ON
L6 SEL L3 1- CHEM : 14 TERMS
SET SMARTSELECT OFF

FILE 'HCAPLUS' ENTERED AT 09:07:35 ON 06 AUG 2003
L7 51560 S L6
L8 53 S L5 (L) L7
L9 48 S L8 AND PD<19990222
L10 1 S L8 (L) PREP/RL
L11 3 S L9 AND PREP/RL

=> d l10 ibib ab

L10 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1996:342147 HCAPLUS
DOCUMENT NUMBER: 125:4414
TITLE: Cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and its use for manufacturing isoleucine and valine
INVENTOR(S): Inui, Masayuki; Man, Tomoko; Kobayashi, Miki; Yugawa, Hideaki
PATENT ASSIGNEE(S): Mitsubishi Chem Corp, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 08089249	A2	19960409	JP 1994-234612	19940929

PRIORITY APPLN. INFO.: JP 1994-234612 19940929

AB The gene encoding dihydroxy-acid dehydratase (E.C. 4.2.1.9) is isolated from Brevibacterium flavum strain MJ-233. Expression plasmid pCRY30-DH encoding the enzyme was prepd. and used for the transformation of coryneform bacteria. Brevibacterium flavum strain MJ-233 transformed with the plasmid produced isoleucine 20 mM into the medium as compared to 10 mM by the wild type.

=> d l11 ibib ab 1-3

L11 ANSWER 1 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1996:342147 HCAPLUS
DOCUMENT NUMBER: 125:4414
TITLE: Cloning of gene for **dihydroxy-acid dehydratase** of coryneform bacteria and its use for manufacturing isoleucine and **valine**
INVENTOR(S): Inui, Masayuki; Man, Tomoko; Kobayashi, Miki; Yugawa, Hideaki
PATENT ASSIGNEE(S): Mitsubishi Chem Corp, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 08089249	A2	19960409	JP 1994-234612	19940929 <--

PRIORITY APPLN. INFO.: JP 1994-234612 19940929
AB The gene encoding dihydroxy-acid dehydratase (E.C. 4.2.1.9) is isolated from Brevibacterium flavum strain MJ-233. Expression plasmid pCRY30-DH encoding the enzyme was prepd. and used for the transformation of coryneform bacteria. Brevibacterium flavum strain MJ-233 transformed with the plasmid produced isoleucine 20 mM into the medium as compared to 10 mM by the wild type.

L11 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1989:150244 HCAPLUS
DOCUMENT NUMBER: 110:150244
TITLE: Purification and inhibition of spinach .alpha.,.beta.-dihydroxyacid dehydratase
AUTHOR(S): Pirrung, Michael C.; Ha, Hyun Joon; Holmes, Christopher P.
CORPORATE SOURCE: Dep. Chem., Stanford Univ., Stanford, CA, 94305, USA
SOURCE: Journal of Organic Chemistry (**1989**), 54(7), 1543-8
CODEN: JOCEAH; ISSN: 0022-3263
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **.alpha.,.beta.-Dihydroxy acid dehydratase** (EC 4.2.1.9) (I), responsible for the prodn. of .alpha.-oxoisovaleric acid in the **valine** biosynthetic pathway, was purified from spinach leaves. Its properties were similar to those given in a previous report using a less pure prepn. The I monomer mol. wt. was estd. to be 55,000. Evidence for an enol intermediate in the I reaction mechanism was obtained by a deuterium labeling study. Several inhibitors of the enzyme were screened. Four that were particularly effective were 4-fluoro-2,3-dihydroxyisovaleric acid, 1-hydroxy-1-isobutanesulfonic acid, N,N-dimethylglycine N-oxide, and 2-fluoro-3,3-dimethylacrylic acid. As an enol analog, the latter compd. gave further evidence for an enol intermediate. Since I was previously found to strongly correlate with plant seedling growth, the possible use of the I inhibitors as herbicides was discussed.

L11 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1971:460228 HCAPLUS
DOCUMENT NUMBER: 75:60228
TITLE: Isoleucine-valine requiring mutants of Salmonella typhimurium. III. Valine-sensitive strains
AUTHOR(S): Armstrong, Frank Bradley; Ishiwa, Hiromi
CORPORATE SOURCE: Dep. Biochem., North Carolina State Univ., Raleigh, NC, USA
SOURCE: Genetics (**1971**), 67(2), 171-82
CODEN: GENTAE; ISSN: 0016-6731
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Enzymic and transductional assays of 4 mutant strains of S. typhimurium which displayed extreme sensitivity to valine provided evidence that acetohydroxy acid synthetase, the 1st enzyme in the common pathway for the synthesis of isoleucine to valine, possessed an increased activity with this amino acid. Valine inhibition of this enzyme also inhibited the induction of reductoisomerase, the 2nd enzyme of the pathway, as well as syntheses of other essential metabolites such as methionine. The phenomenon of valine sensitivity was thought to represent a mutation in the ilv B locus, which represents the structural gene for acetohydroxy acid synthetase. Results of cotransduction tests placed the ilv B locus between the ilv A (threonine dehydratase deficient) and the ilv C (reductoisomerase deficient) loci and furnished the following order for the ilv gene of S. typhimurium: ilvE-ilvD-ilvA-ilvB-ilbC. This order differed from that reported for Escherichia coli, which was ilvE-ilvD-ilvA-ilvC-ilvB.

```
=> d his

     (FILE 'HOME' ENTERED AT 09:35:10 ON 06 AUG 2003)

     FILE 'REGISTRY' ENTERED AT 09:35:16 ON 06 AUG 2003
L1             1 S 72-18-4/RN
L2             1 S 9027-45-6/RN

     FILE 'HCAPLUS' ENTERED AT 09:37:08 ON 06 AUG 2003

     FILE 'REGISTRY' ENTERED AT 09:37:15 ON 06 AUG 2003
                SET SMARTSELECT ON
L3           SEL L1 1- CHEM :       14 TERMS
                SET SMARTSELECT OFF

     FILE 'HCAPLUS' ENTERED AT 09:37:15 ON 06 AUG 2003
L4         51560 S L3

     FILE 'REGISTRY' ENTERED AT 09:37:29 ON 06 AUG 2003
                SET SMARTSELECT ON
L5           SEL L2 1- CHEM :       11 TERMS
                SET SMARTSELECT OFF

     FILE 'HCAPLUS' ENTERED AT 09:37:30 ON 06 AUG 2003
L6          1414 S L5
L7             5 S L6 (L) L4 (L) PREP/RL
L8             3 S L7 AND PD<19990222

     FILE 'REGISTRY' ENTERED AT 10:10:17 ON 06 AUG 2003
L9             1 S 9075-02-9/RN

     FILE 'HCAPLUS' ENTERED AT 10:11:05 ON 06 AUG 2003

     FILE 'REGISTRY' ENTERED AT 10:11:13 ON 06 AUG 2003
                SET SMARTSELECT ON
L10          SEL L9 1- CHEM :       16 TERMS
                SET SMARTSELECT OFF

     FILE 'HCAPLUS' ENTERED AT 10:11:13 ON 06 AUG 2003
L11          311 S L10
L12           21 S L4 (L) L6 (L) L11
L13            0 S L12 (L) PREP/RL
L14           19 S L12 AND PD<19990222
                E CORYNEFORM/CT
                E E3+ALL
                E E1+ALL
                E CORYNEFORM/CT
                E E5+ALL
```

iNBN and iNC

=> d ibib ab 1-19

L14 ANSWER 1 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1996:98247 HCAPLUS
DOCUMENT NUMBER: 124:140640
TITLE: Metabolic effects of inhibitors of two enzymes of the branched-chain amino acid pathway in Salmonella typhimurium
AUTHOR(S): Epelbaum, Sabine; Chipman, David M.; Barak, Ze'ev
CORPORATE SOURCE: Dep. Life Sci., Ben-Gurion Univ. Negev, Beer Sheva, 84105, Israel
SOURCE: Journal of Bacteriology (**1996**), 178(4), 1187-96
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The metabolic effects of inhibitors of 2 enzymes in the pathway for biosynthesis of branched-chain amino acids were examd. in S. typhimurium mutant strain TV105, expressing a single isoenzyme of **acetohydroxy acid synthase** (AHAS), AHAS isoenzyme II. One inhibitor was the sulfonylurea herbicide sulfometuron Me (SMM), which inhibits this isoenzyme and AHAS of other organisms, and the other was N-iso-Pr oxalylhydroxamate (IpOHA), which inhibits **ketol-acid reductoisomerase** (KARI). The effects of the inhibitors on growth, level of several enzymes of the pathway, and levels of intermediates of the pathway were measured. The intracellular concn. of the AHAS substrate 2-ketobutyrate increased on addn. of SMM, but a lack of correlation between increased ketobutyrate and growth inhibition suggests that the former is not the immediate cause of the latter. The levels of the keto acid precursor of **valine**, but not of the precursor of isoleucine, were drastically decreased by SMM, and **valine**, but not isoleucine, partially overcame SMM inhibition. This apparent stronger effect of SMM on the flux into the **valine** arm, as opposed to the isoleucine arm, of the branched-chain amino acid pathway is explained by the kinetics of the AHAS reaction, as well as by the different roles of pyruvate, ketobutyrate, and the **valine** precursor in metab. The organization of the pathway thus potentiates the inhibitory effect of SMM. IpOHA has strong initial effects at lower concns. than does SMM and leads to increases both in the acetohydroxy acid substrates of KARI and, surprisingly, in ketobutyrate. **Valine** completely protected strain TV105 from IpOHA and the MIC. A no. of explanations for this effect can be ruled out, so that some unknown arrangement of the enzymes involved must be suggested. IpOHA led to initial cessation of growth, with partial recovery after a time whose duration increased with the inhibitor concn. The recovery is apparently due to induction of a new KARI synthesis, as well as disappearance of IpOHA from the medium.

L14 ANSWER 2 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:961953 HCAPLUS
DOCUMENT NUMBER: 124:77988
TITLE: Cloning, sequencing and expression of the ilvBNC gene cluster from Streptomyces avermitilis
AUTHOR(S): De Rossi, Edda; Leva, Raffaella; Gusberti, Laura; Manachini, Pier Luigi; Riccardi, Giovanna
CORPORATE SOURCE: Department of Genetics and Microbiology, University of Pavia, 27100, Pavia, Italy
SOURCE: Gene (**1995**), 166(1), 127-32
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The metab. of the branched-chain amino acids (BCAA) isoleucine, leucine and **valine** is correlated to the prodn. of polyketide antibiotics in many streptomycetes. Despite its significance, this biosynthetic pathway is poorly understood in Streptomyces. In order to develop a better understanding of Streptomyces BCCA biosynthesis, two genes, ilvBN and ilvC, encoding **acetohydroxy acid synthase** (AHS) and **acetohydroxy acid isomeroreductase**

(IR), resp., were cloned from Streptomyces avermitilis, a strain producing avermectins, potent antiparasitic compds. The genes were isolated by applying a combination of PCR and genomic library screening. The deduced amino-acid sequences revealed significant homol. to the AHS and IR proteins from other bacterial species. The ilvBN gene, expressed in Escherichia coli (Ec) by using the expression vector pGEX-4T-1, complemented the ilv- mutation of Ec PS1283. Ec transformants produced high levels of AHS, whose activity was feedback inhibited by **valine**.

L14 ANSWER 3 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:599376 HCAPLUS
DOCUMENT NUMBER: 121:199376
TITLE: Identification and sequence determination of the acetohydroxy acid isomeroreductase gene from Brevibacterium flavum MJ233
AUTHOR(S): Inui, Masayuki; Vertes, Alain A.; Kobayashi, Miki; Kurusu, Yasurou; Yukawa, Hideaki
CORPORATE SOURCE: Tsukuba Res. Cent., Mitsubishi Petrochem. Co., Ltd., Inashiki, 300-03, Japan
SOURCE: DNA Sequence (**1993**), 4(2), 95-103
CODEN: DNSEES; ISSN: 1042-5179
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The enzyme **acetohydroxy acid isomeroreductase** (AHAIR) is the second enzyme in the parallel isoleucine-**valine** biosynthetic pathway. The authors previously reported the cloning and sequencing of the **acetohydroxy acid synthase** (AHAS) genes from Brevibacterium flavum MJ233. Anal. of the sequence downstream of the AHAS genes identified another open reading frame highly homologous at the amino acid level to the AHAIR gene from Escherichia coli (ilvC). The authors subcloned the B. flavum AHAIR gene on a 2.1 kb BglII-EcoRI fragment by complementation of an E. coli ilvC mutant. The nucleotide sequence of the B. flavum AHAIR gene consists of 338 codons (mol. wt. of 36158). Comparison of the deduced protein sequence revealed a high degree of identity with the sequences of ilvC genes from other organisms. Disruption of the B. flavum ilvC gene by a kanamycin resistance cassette resulted in L-isoleucine and **L-valine** auxotrophy.

L14 ANSWER 4 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:476041 HCAPLUS
DOCUMENT NUMBER: 121:76041
TITLE: Inhibition of plant acetolactate synthase by herbicides, pyrimidinylsalicylic acids
AUTHOR(S): Shimizu, Tsutomu; Nakayama, Ishizue; Nakao, Tohru; Nezu, Yukio; Abe, Hiroshi
CORPORATE SOURCE: Life Sci. Res. Inst., Kumiai Chem. Ind. Co., Ltd., Ogawa, 439, Japan
SOURCE: Journal of Pesticide Science (International Edition) (**1994**), 19(1), 59-67
CODEN: JPESEC; ISSN: 0916-9962
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The growth inhibition of rice seedlings and chlorella by the herbicides, pyrimidinyl-salicylic acids (PS's) represented by pyrithiobac (2-chloro-6-(4,6-dimethoxpyrimidine-2-ylthio) benzoic acid) was alleviated almost completely by simultaneous application of three branched-chain amino acids, namely leucine, **valine** and isoleucine. PS's inhibited potently **acetolactate synthase** (EC 4.1.3.18) in various species of plants including crops and weeds, which catalyzes the condensation step of two mols. of pyruvate to form acetolactate in the biosynthetic pathway of branched-chain amino acids. PS's, however, affected neither **ketol-acid reductoisomerase** which catalyzes the next reaction step from **acetolactate synthase** in the pathway nor direct acetoin forming enzyme deduced as pyruvate decarboxylase. PS's showed no inhibitory effect on photosynthetic electron transport system of pea, while they inhibited slightly chlorophyll biosynthesis of cotton cotyledons. In spite of high

selectivity of pyrithiobac for cotton plants, there was no difference in sensitivity of ALS to pyrithiobac between cotton and other plants.

L14 ANSWER 5 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:1848 HCAPLUS
DOCUMENT NUMBER: 120:1848
TITLE: Isoleucine synthesis in Corynebacterium glutamicum: molecular analysis of the ilvB-ilvN-ilvC operon
AUTHOR(S): Keilhauer, Carmen; Eggeling, Lothar; Sahm, Hermann
CORPORATE SOURCE: Inst. Biotechnol., Forschungszent., Juelich, D-52425, Germany
SOURCE: Journal of Bacteriology (**1993**), 175(17), 5595-603
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Acetohydroxy acid synthase** (AHAS) and **isomeroreductase** (IR) catalyze subsequent reactions in the flux of metabolites towards isoleucine, **valine**, leucine, and pantothenate. A 4705-bp DNA fragment from Corynebacterium glutamicum known to code for AHAS and IR was sequenced and analyzed by Northern (RNA blot) anal. As in other bacteria, the AHAS of this gram-pos. organism is encoded by two genes, ilvB and ilvN. Gene disruption verified that these genes encode the single AHAS activity in C. glutamicum. The start of ilvB was detd. by amino-terminal sequencing of a fusion peptide. By Northern anal. of the ilvBNC cluster, three in vivo transcripts of 3.9, 2.3, and 1.1 kb were identified, corresponding to ilvBNC, ilvNC, and ilvC messages, resp. The ilvC transcript (encoding IR) was by far the most abundant one. With a clone from which the ilvB upstream regions had been deleted, only the ilvNC and ilvC transcripts were synthesized, and with a clone from which the ilvN upstream regions had been deleted, only the smallest ilvC transcript was formed. It is therefore concluded that in the ilv operon of C. glutamicum, three promoters are active. The amts. of the ilvBNC and ilvNC transcripts increased in response to the addn. of .alpha.-ketobutyrate to the growth medium. This was correlated to an increase in specific AHAS activity, whereas IR activity was not increased because of the relatively large amt. of the ilvC transcript present under all conditions assayed. Therefore, the steady-state level of the ilvBNC and ilvNC messages contributes significantly to the total activity of the single AHAS. The ilvC transcript of this operon, however, is regulated independently and present in a large excess, which is in accord with the const. IR activities detd.

L14 ANSWER 6 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1987:528279 HCAPLUS
DOCUMENT NUMBER: 107:128279
TITLE: Structural heterozygosis at genes ILV2 and ILV5 in Saccharomyces carlsbergensis
AUTHOR(S): Petersen, J. G. L.; Nilsson-Tillgren, T.; Kielland-Brandt, M. C.; Gjermansen, C.; Holmberg, S.
CORPORATE SOURCE: Dep. Physiol., Carlsberg Lab., Copenhagen, DK-2500, Den.
SOURCE: Current Genetics (**1987**), 12(3), 167-74
CODEN: CUGED5; ISSN: 0172-8083
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Chromosomes XII and XIII of a S. calsbergensis brewing strain were analyzed after their transfer into S. cerevisiae by kar1-mediated single chromosome transfer. The lager yeast was found to be heterozygous for the isoleucine-**valine** biosynthesis genes ILV2 (encoding **acetohydroxy acid synthase**) and ILV5 (encoding **acetohydroxy acid reductoisomerase**). In both cases, Southern anal. showed restriction site polymorphisms and that one allele hybridizes more strongly to that of S. cerevisiae than the other. The alleles with limited nucleotide sequence homol. are located on chromosomes which recombine poorly with the corresponding S. cerevisiae chromosomes (XIII and XII) during meiosis. A cluster of rRNA genes is located on chromosome XII with the S. cerevisiae-like ILV5, but not on the homologous chromosome. The present anal. supports the view that S.

carlsbergensis is an amphiploid hybrid.

L14 ANSWER 7 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1983:502048 HCAPLUS
DOCUMENT NUMBER: 99:102048
TITLE: Regulation of the ilv operon in Escherichia coli K-12. Role of Thr and Ile
AUTHOR(S): Okada, Toshihiko
CORPORATE SOURCE: Dep. Biochem., Kanazawa Med. Univ., Uchinada, Japan
SOURCE: Japanese Journal of Genetics (**1983**), 58(1), 59-64
CODEN: IDZAAW; ISSN: 0021-504X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Under nutrient shift-down conditions, threonine (Thr), .alpha.-ketobutyrate, and isoleucine (Ile) derepressed the formation of **acetohydroxy acid synthase** and **isomeroreductase**, but not that of transaminase B or threonine deaminase, in E. coli. Leucine and(or) **valine**, however, reversed the isoleucine-induced derepression. In the absence of isoleucine, leucine and **valine** completely inhibited the derepression of the enzymes after shift-down. Apparently, isoleucine is necessary for the efficient formation of **acetohydroxy acid synthase** isoenzymes I (encoded by gene ilvB) and III (encoded by gene ilv HI).

L14 ANSWER 8 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1983:85943 HCAPLUS
DOCUMENT NUMBER: 98:85943
TITLE: Role of acetohydroxy acid isomeroreductase in biosynthesis of pantothenic acid in Salmonella typhimurium
AUTHOR(S): Primerano, Donald A.; Burns, R. O.
CORPORATE SOURCE: Sch. Med., Duke Univ., Durham, NC, 27710, USA
SOURCE: Journal of Bacteriology (**1983**), 153(1), 259-69
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Structural genes have been identified for all of the enzymes involved in the biosynthesis of pantothenic acid in S. typhimurium and Escherichia coli K-12, with the exception of ketopantoic acid reductase, which catalyzes the conversion of .alpha.-ketopantoate to pantoate. The **acetohydroxy acid isomeroreductase** from S. typhimurium efficiently bound .alpha.-ketopantoate (Km = 0.25 mM) and catalyzed its redn. at 1/20 the rate at which .alpha.-acetolactate was reduced. Since 2 enzymes could apparently participate in the synthesis of pantoate, a S. typhimurium ilvC8 strain was mutagenized to derive strains completely blocked in the conversion of .alpha.-ketopantoate to pantoate. Several isolates were obtained that grew in isoleucine-**valine** medium supplemented with either pantoate or pantothenate, but not in the same medium supplemented with .alpha.-ketopantoate or .beta.-alanine. The mutations that conferred pantoate auxotrophy (designated panE) to these isolates appeared to be clustered, but were not linked to panB or panC. All panE strains tested had greatly reduced levels of ketopantoic acid reductase. The capacity of the **isomeroreductase** to synthesize pantoate in vivo was assessed by detg. the growth requirements of ilvC+ derivs. of panE ilvC8 strains. These strains required either .alpha.-ketopantoate, pantoate, or pantothenate when the **isomeroreductase** was present at low levels; when the synthesis of **isomeroreductase** was induced, panE ilvC+ strains grew in unsupplemented medium. These phenotypes indicate that a high level of **isomeroreductase** is sufficient for the synthesis of pantoate. The panE ilvC+ strains also grew in media supplemented with lysine and methionine. This phenotype resembles that of some S. typhimurium ilvG mutants which are partially blocked in the biosynthesis of CoA and are limited for succinyl CoA. panE ilvC+ Strains which lack the **acetohydroxy acid synthases** required only methionine for growth (in the presence of leucine, isoleucine, and

valine). This and other evidence suggested that the synthesis of pantoic acid by **isomeroreductase** was blocked by the .alpha.-acetohydroxy acids and that pantoic acid synthesis was enhanced in the absence of these intermediates, even when the **isomeroreductase** was at low levels. The panE ilvC+ strains reverted to pantothenate independence. Several of these revertants had elevated **isomeroreductase** levels under noninduced and induced conditions; the suppressing mutation in each revertant was closely linked to ilvC by P22 transduction. This procedure presents a means for obtaining mutants with altered regulation of **isomeroreductase**.

L14 ANSWER 9 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1977:54187 HCAPLUS
DOCUMENT NUMBER: 86:54187
TITLE: A search for **ketol acid reductoisomerase** and **acetolactate synthase** in the tissue of **valine**-fed and **valine**-deprived rats
AUTHOR(S): Koehler, Kathleen M.
CORPORATE SOURCE: Univ. Illinois, Urbana, IL, USA
SOURCE: (**1976**) 230 pp. Avail.: Xerox Univ. Microfilms, Ann Arbor, Mich., Order No. 76-24,120 From: Diss. Abstr. Int. B 1976, 37(5), 2162
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

L14 ANSWER 10 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1976:587335 HCAPLUS
DOCUMENT NUMBER: 85:187335
TITLE: Oxygen and toxicity inhibition of amino acid biosynthesis
AUTHOR(S): Boehme, Daniel E.; Vincent, Kim; Brown, Olen R.
CORPORATE SOURCE: John M. Dalton Res. Cent., Univ. Missouri, Columbia, MO, USA
SOURCE: Nature (London, United Kingdom) (**1976**), 262(5567), 418-20
CODEN: NATUAS; ISSN: 0028-0836
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Escherichia coli E-26 was protected from O toxicity by yeast ext. or by a mixt. of 20 amino acids. Of these, **valine** [**72-18-4**], tyrosine [60-18-4], isoleucine [73-32-5], tryptophan [73-22-3], leucine [61-90-5], phenylalanine [63-91-2], cysteine [52-90-4], methionine [63-68-3], asparagine [70-47-3], and threonine [72-19-5] were essential for full protection. O may therefore inhibit specific enzymes involved in the formation of these essential amino acids; this was studied by adding intermediates of amino acid formation. Hyperbaric O blocked **acetohydroxy acid synthetase**, acetoxy acid **reductoisomerase**, or dihydroxy acid dehydratase, in the parallel paths for leucine, **valine**, and isoleucine formation. The formation of cysteine from serine was impaired, the inhibition occurring before homocysteine. Shikimate [138-59-0] conversion to chorismate [617-12-9] was inhibited.

L14 ANSWER 11 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1975:27748 HCAPLUS
DOCUMENT NUMBER: 82:27748
TITLE: Subcellular localization of isoleucine-valine biosynthetic enzymes in yeast
AUTHOR(S): Ryan, E. D.; Kohlhaw, G. B.
CORPORATE SOURCE: Dep. Biochem., Purdue Univ., West Lafayette, IN, USA
SOURCE: Journal of Bacteriology (**1974**), 120(2), 631-7
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB By using the method of stepwise homogenization of yeast spheroplast lysates employed previously with the leucine biosynthetic enzymes, it was

shown that threonine deaminase, **acetohydroxy acid synthase**, Mg2+-dependent **isomeroreductase**, and dihydroxy acid dehydratase were particulate. Density gradient centrifugation and the behavior of marker enzymes suggest that all of the above enzymes of the isoleucine-**valine** biosynthetic pathway are assocd. with the mitochondria.

L14 ANSWER 12 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1974:435459 HCAPLUS
DOCUMENT NUMBER: 81:35459
TITLE: Role of the leu-3 cistron in the regulation of the synthesis of isoleucine and valine biosynthetic enzymes in Neurospora
AUTHOR(S): Olshan, Arthur R.; Gross, S. R.
CORPORATE SOURCE: Dep. Biochem., Duke Univ., Durham, NC, USA
SOURCE: Journal of Bacteriology (**1974**), 118(2), 374-84
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The prodn. by Neurospora of the enzymes of isoleucine and **valine** synthesis in response to specific end product-derived signals depends upon the presence of an effective leu-3 regulatory product and its effector .alpha.-isopropylmalate (.alpha.-IPM). In leu-3+ strains, threonine deaminase prodn. was repressed as a function of available isoleucine, **acetohydroxy acid synthetase** as a function of **valine**, and the **isomeroreductase** and dihydroxy acid dehydratase as a function of isoleucine and leucine. In the absence of an effective leu-3 regulatory product, .alpha.-isopropylmalate, or both, the prodn. of isoleucine and **valine** biosynthetic enzymes was fixed at or near fully repressed levels even under conditions of severe end product limitation. The leu-3 .alpha.-IPM regulatory product is necessary for full expression of at least 4 genes specifying the structure of the enzymes of isoleucine and **valine** synthesis. The leu-3 .alpha.-IPM regulatory element may facilitate transcription of the genetically dispersed cistrons either by imposing specificity on RNase for structurally similar promoters adjacent to each of the cistrons or by opening promoters after interaction with nearly identical stretches of DNA near each of the structural genes.

L14 ANSWER 13 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1974:142876 HCAPLUS
DOCUMENT NUMBER: 80:142876
TITLE: Biosynthesis of branched-chain amino acids in Schizosaccharomyces pombe. Regulation of the enzymes involved in isoleucine, valine, and leucine synthesis
AUTHOR(S): McDonald, Roderick A.; Satyanarayana, T.; Kaplan, J. G.
CORPORATE SOURCE: Dep. Biol., Univ. Ottawa, Ottawa, ON, Can.
SOURCE: Canadian Journal of Biochemistry (**1974**), 52(1), 51-9
CODEN: CJBIAE; ISSN: 0008-4018
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The activities and regulation of the enzymes of the synthetic pathway of branched-chain amino acids were investigated in S. pombe. The presence in this yeast of threonine deaminase (TD) and **acetohydroxy acid synthetase** (AHAS) is known. The remaining isoleucine-**valine** enzymes, **isomeroreductase** (IR), dehydrase, and transaminase B, were characterized in cell-free exts., indicating the presence of the complete pathway as demonstrated in other microorganisms. .alpha.-Isopropylmalate synthetase (IPMS), the first enzyme of the leucine pathway, has properties of a typical regulatory enzyme. It is most active at pH 7.5-8.5, but is most sensitive to feedback inhibition by L-leucine at pH 6.5-7.0. Unlike baker's yeast, only AHAS and IR appeared to be subject to multivalent repression. TD was relatively resistant to any change in level, and AHAS was repressible by **valine**. IPMS was repressed when cells were grown in complex medium; leucine alone did not cause repression, and in contrast with

baker's yeast, neither did leucine plus threonine nor a combination of all 3 branched-chain amino acids.

L14 ANSWER 14 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1973:156474 HCAPLUS
DOCUMENT NUMBER: 78:156474
TITLE: Isoleucine and valine metabolism in Escherichia coli. XXII. Pleiotropic mutation affecting induction of isometroreductase activity
AUTHOR(S): Pledger, W. J.; Umbarger, H. E.
CORPORATE SOURCE: Dep. Biol. Sci., Purdue Univ., Lafayette, IN, USA
SOURCE: Journal of Bacteriology (**1973**), 114(1), 195-207
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The ilvC gene product, **acetohydroxy acid isomeroreductase**, an enzyme essential for isoleucine and **valine** formation, is subject to substrate induction in E. coli. A mutant of E. coli K-12 with a mutation that renders the ilvC gene product noninducible by its substrates, the acetohydroxy acids, was isolated. This mutation, ilvY466, has been shown to be in a previously undiscovered locus that lies between ilvC and ilvO. The ilvY product, upsilon, is thought to be a regulatory element involved in the induction of ilvC. The recognition site, ilvQ, or upsilon was postulated and was suggested that it lies between ilvC and ilvB. A possible model, involving upsilon, in the pos. control of **isomeroreductase** is presented. Pleiotropic effects of the ilvY466 mutation were recognized from changes in the end-product inhibition of threonine deaminase and of **acetohydroxy acid synthetase**. In addn., pleiotropic effects of this lesion on the regulation of threonine deaminase and the phys. properties of threonine deaminase and **acetohydroxy acid synthetase** were obsd.

L14 ANSWER 15 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1973:156362 HCAPLUS
DOCUMENT NUMBER: 78:156362
TITLE: Isoleucine and valine metabolism in Escherichia coli. XXI. Mutations affecting derepression and valine resistance
AUTHOR(S): Pledger, W. J.; Umbarger, H. E.
CORPORATE SOURCE: Dep. Biol. Sci., Purdue Univ., Lafayette, IN, USA
SOURCE: Journal of Bacteriology (**1973**), 114(1), 183-94
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The activity of **acetohydroxy acid isomeroreductase**, an essential enzyme for isoleucine and **valine** biosynthesis in E. coli, was examd. in a series of mutants contg. derepressed levels of **acetohydroxy acid synthetase** activity but which differed from each other in the sensitivity of the synthetases to **valine** inhibition. The finding that **isomeroreductase** was highest in the strain with the synthetase that was least sensitive to **valine** inhibition supported the model of internal induction of the **isomeroreductase** by its acetohydroxy acid substrates. The mutation leading to the **acetohydroxy acid synthetase** least sensitive to **valine** was unlinked to the ilv gene cluster and appeared to result in a synthetase that differed from the normal enzyme in several properties. The locus of this mutation was designed ilvF. The loci leading to derepression were designated azl. A pleiotropic, apparently single-step, mutation was found that led to restoration of end-product sensitivity to the synthetase, loss of end-product sensitivity of threonine deaminase (EC 4.2.1.16, L-threonine hydrolyase (deaminating)) and loss of **isomeroreductase** activity.

L14 ANSWER 16 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1971:460228 HCAPLUS

DOCUMENT NUMBER: 75:60228
TITLE: Isoleucine-valine requiring mutants of Salmonella typhimurium. III. Valine-sensitive strains
AUTHOR(S): Armstrong, Frank Bradley; Ishiwa, Hiromi
CORPORATE SOURCE: Dep. Biochem., North Carolina State Univ., Raleigh, NC, USA
SOURCE: Genetics (**1971**), 67(2), 171-82
CODEN: GENTAE; ISSN: 0016-6731
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Enzymic and transductional assays of 4 mutant strains of S. typhimurium which displayed extreme sensitivity to **valine** provided evidence that **acetohydroxy acid synthetase**, the 1st enzyme in the common pathway for the synthesis of isoleucine to **valine**, possessed an increased activity with this amino acid. **Valine** inhibition of this enzyme also inhibited the induction of **reductoisomerase**, the 2nd enzyme of the pathway, as well as syntheses of other essential metabolites such as methionine. The phenomenon of **valine** sensitivity was thought to represent a mutation in the ilv B locus, which represents the structural gene for **acetohydroxy acid synthetase**. Results of cotransduction tests placed the ilv B locus between the ilv A (threonine dehydratase deficient) and the ilv C (**reductoisomerase** deficient) loci and furnished the following order for the ilv gene of S. typhimurium: ilvE-ilvD-ilvA-ilvB-ilbC. This order differed from that reported for Escherichia coli, which was ilvE-ilvD-ilvA-ilvC-ilvB.

L14 ANSWER 17 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1970:463483 HCAPLUS
DOCUMENT NUMBER: 73:63483
TITLE: Regulation of isoleucine-valine biosynthesis in Pseudomonas aeruginosa. I. Characterization and mapping of mutants
AUTHOR(S): Marinus, M. G.; Loutit, J. S.
CORPORATE SOURCE: Med. Sch., Univ. Otago, Dunedin, N. Z.
SOURCE: Genetics (**1969**), 63(3), 547-56
CODEN: GENTAE; ISSN: 0016-6731
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Ninety-three isoleucine-**valine** mutants of P. aeruginosa strains 1 and 78 were characterized into 3 functional groups. The group with lesions in the ilvB locus lacked **acetohydroxy acid synthetase**; in the ilvC locus, **reductoisomerase** and the ilvD locus, dihydroxy acid dehydratase. The ilvB and ilvC loci were contiguous, but the ilvD locus mapped at a site almost 25 min from the linked loci, as detd., by conjugation. A tentative fine-structure map of the ilvB gene was constructed by cotransduction of mutants and a **valine**-requiring marker.

L14 ANSWER 18 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1970:29160 HCAPLUS
DOCUMENT NUMBER: 72:29160
TITLE: Metabolism of valine and isoleucine in Escherichia coli. XVII. Role of induction in the depression of acetohydroxy acid isomeroreductase
AUTHOR(S): Arfin, Stuart M.; Ratzkin, Barry; Umbarger, H. Edwin
CORPORATE SOURCE: Purdue Univ., Lafayette, IN, USA
SOURCE: Biochemical and Biophysical Research Communications (**1969**), 37(6), 902-8
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Under conditions that repress the formation of transaminase B, threonine deaminase, dihydroxy acid dehydrase, and **acetohydroxy acid synthetase**, 4 of the 5 enzymes required for isoleucine and **valine** biosynthesis in E. coli, Salmonella typhimurium, and Aerobacter aerogenes (growth with excess isoleucine, **valine**, and leucine), the 5th enzyme, **acetohydroxy acid isomeroreductase**, was induced by its substrates,

acetohydroxy-butyrate and acetolactate. Of the 5 analogs tested, .alpha.-hydroxy-.alpha.-methylbutyrate was the most effective inducer. Substrate induction appears to be the major if not the only means for regulating the formation of this enzyme in these organisms.

L14 ANSWER 19 OF 19 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1965:490534 HCAPLUS
DOCUMENT NUMBER: 63:90534
ORIGINAL REFERENCE NO.: 63:16676h,16677a-b
TITLE: Acetohydroxy acid synthetase in Pseudomonas aeruginosa
AUTHOR(S): Varga, Janos M.; Horvath, Istvan
CORPORATE SOURCE: Res. Inst. Pharm. Chem., Budapest, Hung.
SOURCE: Journal of Molecular Biology (**1965**), 13(2), 596-9
CODEN: JMOBAK; ISSN: 0022-2836
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Cells of P. aeruginosa were harvested after 4 hrs. cultivation and were washed twice in 0.1M K3PO4 buffer (pH 7.6). The sediment (wet wt.) was disrupted in 5 vols. of 0.1M K3PO4 (pH 7.6), 0.1M MgSO4, and 100 .gamma./ml. thiamine pyrophosphate, by ultrasonic treatment. The crude ext. was satd. to 35% with (NH4)2SO4 in 0.5M K3PO4 (pH 7.2). The ppt. was discarded. To the supernatant fraction, (NH4)2SO4 was added to 60% satn. and the ppt. thus formed was suspended in the same soln. used for disruption. After treatment with Sephadex G-25 the soln. was passed through a 1.5 cm. .times. 45 cm. Sephadex G-200 column at a flow rate of 3 ml./hr.; 1.5-ml. fractions were collected. The jacket of the column was cooled with water at 0.degree.. Two sep. fractions (A and B) showing **acetohydroxy acid synthetase** activity were obtained. The two fractions exhibited a marked difference in their sensitivity to **valine**. On storage at 0.degree., the sensitivity to **valine** of fraction A decreased, while that of fraction B increased. The **acetohydroxy acid synthetase** activity of both fractions had the same pH optimum (pH 8). After 10 min. at 37.degree., or 5 consecutive cycles of freezing and thawing, the sensitivity of fraction A to **valine** decreased with a simultaneous increase of its specific activity. It appeared that fraction A and B were aggregations of a group of enzymes (**acetohydroxy acid synthetase**, **reductoisomerase**, dihydroxy acid dehydrase, and transaminase-B), with fraction A the more complex. It is postulated that **acetohydroxy acid synthetase** requires a particular state of aggregation to be active. The simultaneous presence of fractions A and B in the intact bacterial cell would help explain results obtained in growth expts.

=>

=> d ibib ab 1-3

L8 ANSWER 1 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1993:489636 HCAPLUS
DOCUMENT NUMBER: 119:89636
TITLE: Purification and characterization of the valine sensitive acetolactate synthase from Serratia marcescens ATCC 25419
AUTHOR(S): Yang, Jeong Hee; Kim, Soung Soo
CORPORATE SOURCE: Department of Biochemistry, College of Science, Yonsei University, Seoul, S. Korea
SOURCE: Biochimica et Biophysica Acta (**1993**), 1157(2), 178-84
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The valine sensitive acetolactate synthase (ALS) isoenzyme from Serratia marcescens ATCC 25419 was purified to homogeneity. Anal. of the native mol. wt. of the purified enzyme by the native pore gradient polyacrylamide gel electrophoresis indicated the mol. wt. of about 178 000 and sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed the enzyme to be composed of two different types of subunits with mol. wts. of 62,000 and 35,000. The molar ratio of the two polypeptides was estd. to be 1, suggesting that native enzyme is composed of two large subunits and two small subunits. The enzyme exhibits homotropic allosterism with pyruvate unlike other enteric ALS isoenzymes. The specificity ratio R (V[acetohydroxybutyrate]/V[acetolactate] = Rx[.alpha.-ketobutyrate]/[pyruvate]), of the enzyme was found to be 0 suggesting that the Serratia ALS has very high specificity for pyruvate. The pH optimum was around 7.5, and the enzyme was stable at 50.degree.C for 30 min. The pI value for the purified enzyme was 5.2. The concn. of branched chain amino acids for 50% inhibition of the enzyme was 0.1 mM for valine, and 1 mM for leucine and isoleucine, resp.

L8 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1990:513851 HCAPLUS
DOCUMENT NUMBER: 113:113851
TITLE: Recombinant microorganisms containing an acetohydroxy acid synthase-encoding vector for manufacture of amino acids
INVENTOR(S): Sato, Katsuaki; Yoshino, Eriko; Hashiguchi, Kenichi; Enei, Hitoshi
PATENT ASSIGNEE(S): Ajinomoto Co., Inc., Japan
SOURCE: Eur. Pat. Appl., 11 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
EP 356739	A1	19900307	EP 1989-114207	19890801	<--
EP 356739	B1	19951213			
R: DE, FR, NL					
JP 02042988	A2	19900213	JP 1988-194031	19880803	<--
JP 2748418	B2	19980506			

PRIORITY APPLN. INFO.: JP 1988-194031 19880803
AB Plasmids or phage contg. a gene for an acetohydroxy acid synthase (AHAS) are prepd. Microorganisms transformed with these vectors can be used to manuf. valine, isoleucine, or leucine. The AHAS gene of Brevibacterium lactofermentum was cloned. B. lactofermentum or B. flavum was transformed with the resulting plasmid (pAJ200V3). The transformants produced 7 and 11 g valine/L culture medium, resp.

L8 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1984:606512 HCAPLUS
DOCUMENT NUMBER: 101:206512
TITLE: A basal unit of valine-sensitive acetolactate synthase

of Neurospora crassa

AUTHOR(S): Tanaka, Hiroshi; Kuwana, Homare
CORPORATE SOURCE: Fac. Sci., Kwansei Gakuin Univ., Nishinomiya, 662, Japan

SOURCE: Biochemical and Biophysical Research Communications (**1984**), 123(2), 418-23
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The valine sensitivity as well as the activity of acetolactate synthase of N. crassa was stabilized with 1.2M K2HPO4 buffer during extn. from mitochondria and early stages of purifn.; 20% glycerol plus 5 mM Na pyruvate was included during purifn. on Sephadex G200 gel chromatog. The enzyme was expressed as 4 subunits having mol. wts. of .apprx.500,000, 140,000, 68,000, and 51,000, resp. The 1st and the 3rd species showed valine sensitivity, but the 2nd and the 4th did not. The subunit with a mol. wt. of 68,000 may be the basal unit of valine-sensitive acetolactate synthase of N. crassa.

```
> d his

     (FILE 'HOME' ENTERED AT 10:43:45 ON 06 AUG 2003)

     FILE 'REGISTRY' ENTERED AT 10:44:14 ON 06 AUG 2003
L1             1 S 72-18-4/RN

     FILE 'HCAPLUS' ENTERED AT 10:44:36 ON 06 AUG 2003

     FILE 'REGISTRY' ENTERED AT 10:44:40 ON 06 AUG 2003
                SET SMARTSELECT ON
L2           SEL L1 1- CHEM :      14 TERMS
                SET SMARTSELECT OFF

     FILE 'HCAPLUS' ENTERED AT 10:44:40 ON 06 AUG 2003
L3         51560 S L2
L4          2311 S L3 (L) PREP/RL
L5             1 S L4 (L) (INACTIVAT? OR ELIMINAT? OR DELET?)
L6            64 S L4 AND (INACTIVAT? OR ELIMINAT? OR DELET?)
L7            51 S L6 AND PD<19990222

     FILE 'STNGUIDE' ENTERED AT 10:58:02 ON 06 AUG 2003

     FILE 'HCAPLUS' ENTERED AT 11:10:48 ON 06 AUG 2003
```

=> d ibib ab 2

L7 ANSWER 2 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:195478 HCAPLUS
DOCUMENT NUMBER: 130:249283
TITLE: Genetic and physiological analysis of the formation of pantothenate and valine in Corynebacterium glutamicum
AUTHOR(S): Reuter, Uwe
CORPORATE SOURCE: Inst. Biotechnologie, Forschungszentrum Juelich G.m.b.H., Juelich, D-52425, Germany
SOURCE: Berichte des Forschungszentrums Juelich (1998***), Juel-3606, 1-115 pp.
CODEN: FJBEE5; ISSN: 0366-0885
DOCUMENT TYPE: Report
LANGUAGE: German

AB The Gram-pos. bacterium C. glutamicum is used for the prodn. of amino acids, e.g. of L-glutamate and L-Lys. The biosynthetic pathway of pantothenic acid of this organism was elucidated, and the formation of L-Val and D-pantothenic acid to enable a microbiol. prodn. of these compds was increased. The genes panB and panC were cloned, which encode ketopantoate hydroxymethyltransferase and pantothenate synthetase. The 2 enzymes catalyze important steps of the biosynthetic pathway of pantothenate. Sequence anal. revealed that panB comprises 813 bp and panC 837 bp. The genes are organized as an operon. Assays for the enzymes of the pathway were developed. The pantothenate synthetase has a sp. activity of 1 nmol/min-mg protein, ketopantoate hydroxymethyltransferase one of 0.14 nmol/min-mg protein, and the aspartate .alpha.-decarboxylase one of 0.11 nmol/min-mg protein. The quant. anal. of the formation of pantothenic acid revealed that C. glutamicum accumulates 10 .mu.g pantothenic acid/L. A system to isolate mutants with an increased formation of pantothenate, which is based on a deficiency of pantothenic acid induced by .alpha.-ketobutyrate, was established. The application of this method lad to the isolation of a mutant which accumulates 250 .mu.g pantothenate and 1.4 g valine/L. Overexpression of the genes of the valine and isoleucine biosynthetic pathway (ilvBNCD), in combination with the ***deletion of the threonine dehydratase gene ilvA, resulted in the construction of a strain which accumulates 11.3 g valine and 190 mg pantothenate/L. Addnl. overexpression of panBC led to an accumulation of .ltoreq.1 g pantothenic acid/L. Thus, an increase of the formation of pantothenic acid in C. glutamicum by a factor of 105 has been achieved.

REFERENCE COUNT: 163 THERE ARE 163 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d ti 1-51

L7 ANSWER 1 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overproduction

L7 ANSWER 2 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Genetic and physiological analysis of the formation of pantothenate and valine in Corynebacterium glutamicum

L7 ANSWER 3 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI tRNAVal-heterodimeric maxizymes with high potential as gene-**inactivating** agents: Simultaneous cleavage at two sites in HIV-1 tat mRNA in cultured cells

L7 ANSWER 4 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Bacillus .alpha.-amylase variants with improved washing performance

L7 ANSWER 5 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Photochemical desulfurization of L-cysteine derivatives

L7 ANSWER 6 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Bacillus .alpha.-amylase variants with improved washing performance

L7 ANSWER 7 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Preparation of enantiomerically pure D-amino acids by fermentative with transgenic cells

L7 ANSWER 8 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Interleukin-3 muteins with improved biological activity and their recombinant production

L7 ANSWER 9 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Highly Potent Irreversible Inhibitors of Neutrophil Elastase Generated by Selection from a Randomized DNA-Valine Phosphonate Library

L7 ANSWER 10 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Tripeptidylpeptidase inhibitors, methods of synthesis, and use of inhibitors in treatment of gastrointestinal and mental disorders

L7 ANSWER 11 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Purification and characterization of the human pro-apoptotic cysteine proteinase, apopain, and its modulation by peptidyl inhibitors or gene therapy

L7 ANSWER 12 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Polymer- versus Silica-Based Separation Media: **Elimination** of Nonspecific Interactions in the Chiral Recognition Process through Functional Polymer Design

L7 ANSWER 13 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI N-Alkylcarbamoyl derivatives of amino acids as chiral stationary phases for high-performance liquid chromatography. I. An example of enhancing enantioselectivity by **deleting** the non-enantioselective .pi.-.pi. interaction site on the chiral stationary phase

L7 ANSWER 14 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI The effect of compounds related to penicillin G biosynthesis on the in vitro formation and bioassay of isopenicillin N

L7 ANSWER 15 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Asymmetric induction reactions. V. The palladium-catalyzed asymmetric .alpha.-allylation of carbonyl compounds with chiral allyl esters via enamines and imines

L7 ANSWER 16 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Identification and suppression of decomposition during carbodiimide-mediated reactions of Boc amino acids with phenols, hydroxylamines and amino acid ester hydrochlorides

L7 ANSWER 17 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Cysteine scanning mutagenesis of putative transmembrane helixes IX and X in the lactose permease of Escherichia coli

L7 ANSWER 18 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Potent HIV-1 protease inhibitors: stereoselective synthesis of a dipeptide mimic

L7 ANSWER 19 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Synthetic study on peptide antibiotic nisin. V. Total synthesis of nisin

L7 ANSWER 20 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Stereoselective synthesis of (2S,6S)-2,6-diaminoheptanedioic acid and of unsymmetrical derivatives of meso-2,6-diaminoheptanedioic acid

L7 ANSWER 21 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Substrate specificity of isopenicillin N synthase

L7 ANSWER 22 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Studies on chemical synthesis of the lanthionine peptide nisin

L7 ANSWER 23 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Studies related to a convergent fragment-coupling approach to peptide synthesis using the Kaiser oxime resin

L7 ANSWER 24 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Enzymes for preparation of amino acids from 5'-substituted hydantoins or N-carbamoyl-amino acids

L7 ANSWER 25 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Inhibition of human leukocyte elastase (HLE) by N-substituted peptidyl trifluoromethyl ketones

L7 ANSWER 26 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Synthesis of teleocidins A, B and their congeners. Par 3. Synthesis of dihydroteleocidin B-4 (dihydroteleocidin B), teleocidin B-3 and teleocidin B-4

L7 ANSWER 27 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Molecular basis for different rates of recovery from **inactivation** in the Shaker potassium channel family

L7 ANSWER 28 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI In vitro non-thrombogenicity of a thrombin-substrate-immobilized polymer surface by the inhibition of thrombin activity

L7 ANSWER 29 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Stable carbon isotope analysis of amino acid enantiomers by conventional isotope ratio mass spectrometry and combined gas chromatography/isotope ratio mass spectrometry

L7 ANSWER 30 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Preparation of dehydroalanine peptides from bis(2,2,2-trichloroethyl) and diphenyl phosphonoserine derivatives

L7 ANSWER 31 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Preparation of chiral amino acids via hydrocarboxylation of chiral .alpha.-enamides

L7 ANSWER 32 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI O'-(epoxyalkyl)tyrosines and (epoxyalkyl)phenylalanine as irreversible **inactivators** of serine proteases: synthesis and inhibition mechanism

L7 ANSWER 33 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Synthetic approaches to 2-methoxycysteine containing peptides. The conversion of [(5S)-5-amino-5-carboxy-2-oxapentanoyl]-2-methoxy-(2S)-cysteinyl-(2R)-valine into 10-oxa-6.alpha.-methoxyisopenicillin N by the

enzyme isopenicillin N synthase

L7 ANSWER 34 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Hydantoin derivatives. A new class of inhibitors of human leukocyte elastase

L7 ANSWER 35 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Hydrolysis of proteins and peptides in a hermetically sealed microcapillary tube: high recovery of labile amino acids

L7 ANSWER 36 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI A stereoselective synthesis of N-tert-butoxycarbonyl .alpha.-amino alcohols and .alpha.-amino acids

L7 ANSWER 37 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI 1-Isopropylallyloxycarbonyl as a protective group of amines and its deprotection catalyzed by a palladium-phosphine complex

L7 ANSWER 38 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Diisopropylethylamine **eliminates** dipeptide formation during the acylation of amino acids using benzoyl chloride and some alkyl chloroformates

L7 ANSWER 39 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Studies on a model of bitter peptides including arginine, proline and phenylalanine residues. Part IV. Variation in bitterness potency when introducing Gly-Gly residue into bitter peptides

L7 ANSWER 40 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Biogenetic cassette

L7 ANSWER 41 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Amino acid alkyl ester-modified proteins as emulsifiers for cosmetics and as hair protectants

L7 ANSWER 42 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Antineoplastic agents. 109. Structural biochemistry. 24. Synthesis of the cyclo-[(gly)Thz-(R)- and (S)-(gln)Thz-L-Val-L-Leu-L-Pro] isomers of dolastatin 3

L7 ANSWER 43 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI 2(1H)-Pyridone as leaving group in acylation reactions - applications in peptide chemistry

L7 ANSWER 44 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Amino acids and peptides. XII. Synthesis of C-terminal decapeptide of bovine pancreatic ribonuclease A (RNase A) and its analogs and determination of their ability to reactivate Des(121-124) RNase A

L7 ANSWER 45 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Synthesis and structure of a peptide antibiotic, TL-119 and/or A-3302-B

L7 ANSWER 46 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Synthetic studies on detoxin. The absolute stereochemistry of detoxinine, an unusual .beta.-hydroxy-.gamma.-imino acid

L7 ANSWER 47 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Regeneration of activity by mixture of ribonuclease enzymically degraded from the carboxy-end and a synthetic carboxy-terminal tetradecapeptide

L7 ANSWER 48 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Reaction of uranium with some biocomplexons

L7 ANSWER 49 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Studies on polypeptides. XLI. The synthesis of [5-valine,6-.beta.-(pyrazolyl-3)-alanine]-angiotensin II, a potent hypertensive peptide

L7 ANSWER 50 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Polarographic study of the redox and complexation reactions of

penicillamine, N-acetylpenicillamine, and copper(I) or copper(II) ions

L7 ANSWER 51 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Preparation and some properties of peptidyl derivatives of transfer ribonucleic acid

L7 ANSWER 1 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:302513 HCAPLUS
DOCUMENT NUMBER: 131:85307
TITLE: D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overproduction
AUTHOR(S): Sahm, Hermann; Eggeling, Lothar
CORPORATE SOURCE: Institut fur Biotechnologie, Forschungszentrum Julich GmbH, Julich, 52425, Germany
SOURCE: Applied and Environmental Microbiology (**1999**), 65(5), 1973-1979
CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
CLASSIFICATION: 10-2 (Microbial, Algal, and Fungal Biochemistry)

Inventors Paper

ABSTRACT:
D-Pantothenate is synthesized via four enzymes from ketoisovalerate, which is an intermediate of branched-chain amino acid synthesis. We quantified three of these enzyme activities in Corynebacterium glutamicum and detd. specific activities ranging from 0.00014 to 0.001 .mu.mol/min mg (protein)-1. The genes encoding the ketopantoate hydroxymethyltransferase and the pantothenate synthetase were cloned, sequenced, and functionally characterized. These studies suggest that panBC constitutes an operon. By using panC, an assay system was developed to quantify D-pantothenate. The wild type of C. glutamicum was found to accumulate 9 .mu.g of this vitamin per L. A strain was constructed (i) to abolish L-isoleucine synthesis, (ii) to result in increased ketoisovalerate formation, and (iii) to enable its further conversion to D-pantothenate. The best resulting strain has ilvA **deleted** from its chromosome and has two plasmids to overexpress genes of ketoisovalerate (ilvBNCD) and D-pantothenate (panBC) synthesis. With this strain a D-pantothenate accumulation of up to 1 g/L is achieved, which is a 105-fold increase in concn. compared to that of the original wild-type strain. From the series of strains analyzed it follows that an increased ketoisovalerate availability is mandatory to direct the metabolite flux into the D-pantothenate-specific part of the pathway and that the availability of .beta.-alanine is essential for D-pantothenate formation.

SUPPL. TERM: pantothenate formation Corynebacterium valine gene panBC
INDEX TERM: Gene, microbial
ROLE: PRP (Properties)
(panB; D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overprodn.)
INDEX TERM: Gene, microbial
ROLE: PRP (Properties)
(panC; D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overprodn.)
INDEX TERM: Corynebacterium glutamicum
DNA sequences
Protein sequences
(D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overprodn.)
INDEX TERM: 221625-00-9, Hydroxymethyltransferase, ketopantoate (Corynebacterium glutamicum strain ATCC 13032 gene panB)
221626-41-1, Synthetase, pantothenate (Corynebacterium glutamicum strain ATCC 13032 gene panC)
ROLE: PRP (Properties)
(amino acid sequence; D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overprodn.)
INDEX TERM: 221624-99-3, GenBank X96580
ROLE: PRP (Properties)
(nucleotide sequence; D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate

overprodn.)

INDEX TERM: 9024-57-1, Aspartate decarboxylase
ROLE: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overprodn.)

INDEX TERM: 9023-49-8, Pantothenate synthetase 56093-17-5, Ketopantoate hydroxymethyltransferase
ROLE: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overprodn.)

INDEX TERM: **72-18-4P**, **L-Valine**, preparation
79-83-4P
ROLE: BPN (Biosynthetic preparation); BIOL (Biological study); **PREP (Preparation)**
(D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding **L-valine** synthesis for D-pantothenate overprodn.)

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD.

REFERENCE(S):

(1) Baigori, M; J Bacteriol 1991, V173, P4240 HCAPLUS
(2) Cordes, C; Gene 1992, V112, P113 HCAPLUS
(3) Cremer, J; Mol Gen Genet 1990, V220, P478 HCAPLUS
(4) Cronan, J; J Bacteriol 1980, V141, P1291 HCAPLUS
(5) Cronan, J; J Bacteriol 1982, V149, P916 HCAPLUS
(6) Eggeling, I; Appl Microbiol Biotechnol 1987, V25, P346 HCAPLUS
(7) Eggeling, L; Appl Microbiol Biotechnol 1997, V49, P24
(8) Eggeling, L; J Biotechnol 1997, V56, P167 HCAPLUS
(9) Eikmanns, B; Gene 1991, V102, P93 HCAPLUS
(10) Eikmanns, B; J Bacteriol 1992, V174, P6076 HCAPLUS
(11) Frodyma, M; J Biol Chem 1998, V273, P5572 HCAPLUS
(12) Hara, S; Anal Chim Acta 1985, V172, P167 HCAPLUS
(13) Hatakeyama, K; DNA Sequence 1993, V4, P177 HCAPLUS
(14) Jackowski, S; Escherichia coli and Salmonella:cellular and molecular biology, 2nd ed 1996
(15) Jackowski, S; J Bacteriol 1981, V146, P926
(16) Jager, W; J Bacteriol 1992, V174, P5462 MEDLINE
(17) Jones, C; J Bacteriol 1993, V175, P2125 HCAPLUS
(18) Julliard, J; Bot Acta 1994, V107, P191 HCAPLUS
(19) Kataoka, M; Appl Microbiol Biotechnol 1995, V44, P333 HCAPLUS
(20) Keilhauer, C; J Bacteriol 1993, V175, P5595 HCAPLUS
(21) Konstantinov, K; J Ferment Bioeng 1991, V71, P350 HCAPLUS
(22) Leuchtenberger, W; Biotechnology 1996, V6 HCAPLUS
(23) Liebl, W; FEMS Microbiol Lett 1989, V65, P299 HCAPLUS
(24) Lindroth, P; Anal Chem 1979, V51, P1167
(25) Marx, A; Biotechnol Bioeng 1996, V49, P111
(26) Marx, A; Biotechnol Bioeng 1997, V56, P168 HCAPLUS
(27) Menkel, E; Appl Environ Microbiol 1989, V55, P684 HCAPLUS
(28) Merkel, W; FEMS Microbiol Lett 1996, V143, P247 HCAPLUS
(29) Miyatake, K; J Biochem 1976, V79, P673 HCAPLUS
(30) Mockel, B; Mol Microbiol 1994, V13, P833 MEDLINE
(31) Ondrejkova, A; Unpublished data
(32) Oppenheim, D; Genetics 1980, V95, P785 MEDLINE
(33) Patek, M; Appl Environ Microbiol 1994, V60, P133 HCAPLUS
(34) Powers, S; J Biol Chem 1976, V251, P3786 HCAPLUS
(35) Primerano, D; J Bacteriol 1982, V150, P1202 HCAPLUS
(36) Ramjee, M; Biochem J 1997, V323, P661 HCAPLUS
(37) Sahm, H; FEMS Microbiol Rev 1995, V16, P243 HCAPLUS
(38) Santamaria, R; J Bacteriol 1985, V162, P463 HCAPLUS

(39) Schafer, A; Gene 1994, V145, P69 MEDLINE
(40) Schafer, A; J Bacteriol 1990, V172, P1663 MEDLINE
(41) Schell, M; Annu Rev Microbiol 1993, V47, P597 HCAPLUS
(42) Shimizu, S; Appl Environ Microbiol 1984, V48, P1118 HCAPLUS
(43) Shimizu, S; Opportunities with industrial enzymes 1992
(44) Simon, R; Bio/Technology 1983, V1, P784 HCAPLUS
(45) Sorokin, A; Microbiology 1996, V142, P2005 HCAPLUS
(46) Tanaka, S; J Bacteriol 1967, V93, P642 HCAPLUS
(47) Teller, J; J Biol Chem 1976, V251, P3780 HCAPLUS
(48) Vallari, D; J Bacteriol 1985, V162, P1156 HCAPLUS
(49) Vandamme, E; J Chem Technol Biotechnol 1992, V53, P313 HCAPLUS
(50) Williamson, J; J Biol Chem 1979, V254, P8074 HCAPLUS

```
=> d his

     (FILE 'HOME' ENTERED AT 11:37:00 ON 06 AUG 2003)

     FILE 'REGISTRY' ENTERED AT 11:37:54 ON 06 AUG 2003
L1             1 S 56093-17-5/RN
L2             1 S 9023-49-8/RN
L3             1 S 72-18-4/RN

     FILE 'HCAPLUS' ENTERED AT 11:40:43 ON 06 AUG 2003

     FILE 'REGISTRY' ENTERED AT 11:40:53 ON 06 AUG 2003
                SET SMARTSELECT ON
L4           SEL L1 1- CHEM :       7 TERMS
                SET SMARTSELECT OFF

     FILE 'HCAPLUS' ENTERED AT 11:40:53 ON 06 AUG 2003
L5            62 S L4

     FILE 'REGISTRY' ENTERED AT 11:40:57 ON 06 AUG 2003
                SET SMARTSELECT ON
L6           SEL L2 1- CHEM :       6 TERMS
                SET SMARTSELECT OFF

     FILE 'HCAPLUS' ENTERED AT 11:40:57 ON 06 AUG 2003
L7            68 S L6

     FILE 'REGISTRY' ENTERED AT 11:40:58 ON 06 AUG 2003
                SET SMARTSELECT ON
L8           SEL L3 1- CHEM :      14 TERMS
                SET SMARTSELECT OFF

     FILE 'HCAPLUS' ENTERED AT 11:40:59 ON 06 AUG 2003
L9         51560 S L8
L10            2 S L9 (L) L7 (L) L5
L11            6 S L9 (L) L7
L12            7 S L5 (L) (DELET? OR INACTIVAT? OR ELIMINAT?)
L13            3 S L12 AND PD<19990222
L14            4 S L7 (L) (DELET? OR INACTIVAT? OR ELIMINAT?)
L15            9 S  PANB (L) (DELET? OR INACTIVAT? OR ELIMINAT?)
L16            3 S L15 AND PD<19990222
L17           34 S  PANC (L) (DELET? OR INACTIVAT? OR ELIMINAT?)
L18           14 S L17 AND PD<19990222
```

```
=> d his

     (FILE 'HOME' ENTERED AT 11:37:00 ON 06 AUG 2003)

     FILE 'REGISTRY' ENTERED AT 11:37:54 ON 06 AUG 2003
L1             1 S 56093-17-5/RN
L2             1 S 9023-49-8/RN
L3             1 S 72-18-4/RN

     FILE 'HCAPLUS' ENTERED AT 11:40:43 ON 06 AUG 2003

     FILE 'REGISTRY' ENTERED AT 11:40:53 ON 06 AUG 2003
                SET SMARTSELECT ON
L4           SEL L1 1- CHEM :        7 TERMS
                SET SMARTSELECT OFF

     FILE 'HCAPLUS' ENTERED AT 11:40:53 ON 06 AUG 2003
L5            62 S L4

     FILE 'REGISTRY' ENTERED AT 11:40:57 ON 06 AUG 2003
                SET SMARTSELECT ON
L6           SEL L2 1- CHEM :        6 TERMS
                SET SMARTSELECT OFF

     FILE 'HCAPLUS' ENTERED AT 11:40:57 ON 06 AUG 2003
L7            68 S L6

     FILE 'REGISTRY' ENTERED AT 11:40:58 ON 06 AUG 2003
                SET SMARTSELECT ON
L8           SEL L3 1- CHEM :       14 TERMS
                SET SMARTSELECT OFF

     FILE 'HCAPLUS' ENTERED AT 11:40:59 ON 06 AUG 2003
L9         51560 S L8
L10            2 S L9 (L) L7 (L) L5
L11            6 S L9 (L) L7
L12            7 S L5 (L) (DELET? OR INACTIVAT? OR ELIMINAT?)
L13            3 S L12 AND PD<19990222
L14            4 S L7 (L) (DELET? OR INACTIVAT? OR ELIMINAT?)
L15            9 S  PANB (L) (DELET? OR INACTIVAT? OR ELIMINAT?)
L16            3 S L15 AND PD<19990222
L17           34 S  PANC (L) (DELET? OR INACTIVAT? OR ELIMINAT?)
L18           14 S L17 AND PD<19990222
```

L12 ANSWER 1 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:276126 HCAPLUS
DOCUMENT NUMBER: 136:308622
TITLE: Process for the fermentative preparation of D-pantothenic acid using coryneform bacteria with poxb gene being eliminated
INVENTOR(S): Dusch, Nicole; Hermann, Thomas; Thierbach, Georg
PATENT ASSIGNEE(S): Degussa A.-G., Germany
SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002029020	A1	20020411	WO 2001-EP10212	20010905
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
DE 10117085	A1	20020411	DE 2001-10117085	20010406
AU 2001091825	A5	20020415	AU 2001-91825	20010905
EP 1320586	A1	20030625	EP 2001-972003	20010905
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2002150999	A1	20021017	US 2001-965825	20011001

PRIORITY APPLN. INFO.:
DE 2000-10048604 A 20000930
DE 2001-10117085 A 20010406
DE 2000-10047142 A 20000923
WO 2001-EP10212 W 20010905

AB The invention provides a process for the improved fermentative prepn. of D-pantothenic acid using coryneform bacteria in which the the nucleotide sequence of poxB gene which codes for pyruvate oxidase (EC 1.2.2.2) is attenuated, in particular eliminated. The following steps being carried out: (a) fermn. of D-pantothenic acid-producing bacteria in which at least the gene which codes for pyruvate oxidase PoxB is attenuated; (b) concn. of the D-pantothenic acid in the medium or in the cells of the bacteria; and (c) isolation of the D-pantothenic acid produced. The strains employed optionally already produce D-pantothenic acid before attenuation of the poxB gene. A new nucleotide sequences, which lie upstream and downstream of the poxB gene region have been found. It has been found that these polynucleotides are useful in the prodn. of mutants with an attenuated, in particular eliminated, poxB gene. It has also been found that coryneform bacteria produce pantothenic acid in an improved manner after attenuation of the poxB gene.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 2 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:886541 HCAPLUS
DOCUMENT NUMBER: 136:36479
TITLE: A process for the fermentative preparation of D-pantothenic acid using coryneform bacteria with deleted pck (phosphoenolpyruvate carboxykinase, 4.1.1.49) gene
INVENTOR(S): Dusch, Nicole; Thierbach, Georg
PATENT ASSIGNEE(S): Degussa Ag, Germany
SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1

L12 ANSWER 1 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:276126 HCAPLUS
DOCUMENT NUMBER: 136:308622
TITLE: Process for the fermentative preparation of D-pantothenic acid using coryneform bacteria with poxb gene being eliminated
INVENTOR(S): Dusch, Nicole; Hermann, Thomas; Thierbach, Georg
PATENT ASSIGNEE(S): Degussa A.-G., Germany
SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002029020	A1	20020411	WO 2001-EP10212	20010905
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
DE 10117085	A1	20020411	DE 2001-10117085	20010406
AU 2001091825	A5	20020415	AU 2001-91825	20010905
EP 1320586	A1	20030625	EP 2001-972003	20010905
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2002150999	A1	20021017	US 2001-965825	20011001

PRIORITY APPLN. INFO.: DE 2000-10048604 A 20000930
DE 2001-10117085 A 20010406
DE 2000-10047142 A 20000923
WO 2001-EP10212 W 20010905

AB The invention provides a process for the improved fermentative prepn. of D-pantothenic acid using coryneform bacteria in which the the nucleotide sequence of poxB gene which codes for pyruvate oxidase (EC 1.2.2.2) is attenuated, in particular eliminated. The following steps being carried out: (a) fermn. of D-pantothenic acid-producing bacteria in which at least the gene which codes for pyruvate oxidase PoxB is attenuated; (b) concn. of the D-pantothenic acid in the medium or in the cells of the bacteria; and (c) isolation of the D-pantothenic acid produced. The strains employed optionally already produce D-pantothenic acid before attenuation of the poxB gene. A new nucleotide sequences, which lie upstream and downstream of the poxB gene region have been found. It has been found that these polynucleotides are useful in the prodn. of mutants with an attenuated, in particular eliminated, poxB gene. It has also been found that coryneform bacteria produce pantothenic acid in an improved manner after attenuation of the poxB gene.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 2 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:886541 HCAPLUS
DOCUMENT NUMBER: 136:36479
TITLE: A process for the fermentative preparation of D-pantothenic acid using coryneform bacteria with deleted pck (phosphoenolpyruvate carboxykinase, 4.1.1.49) gene
INVENTOR(S): Dusch, Nicole; Thierbach, Georg
PATENT ASSIGNEE(S): Degussa Ag, Germany
SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001092556	A1	20011206	WO 2001-EP4816	20010428
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
DE 10026758	A1	20011206	DE 2000-10026758	20000530
EP 1285083	A1	20030226	EP 2001-940381	20010428
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2002042104	A1	20020411	US 2001-852118	20010510

PRIORITY APPLN. INFO.: DE 2000-10026758 A 20000530
WO 2001-EP4816 W 20010428

AB The invention provides a process for prepg. D-pantothenic acid by the fermn. of coryneform bacteria in which bacteria are used in which the nucleotide sequence (pck gene) coding for phosphoenolpyruvate carboxykinase (EC 4.1.1.49) is attenuated, wherein the following steps are performed: fermn. of D-pantothenic acid producing bacteria in which at least the gene coding for phosphoenolpyruvate carboxykinase is attenuated, enrichment of D-pantothenic acid in the medium or in the cells of the bacteria, and isolation of the D-pantothenic acid produced.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 3 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:169488 HCAPLUS
DOCUMENT NUMBER: 135:252592
TITLE: Molecular cloning and application of a gene complementing pantothenic acid auxotrophy of sake yeast Kyokai no. 7
AUTHOR(S): Shimoi, Hitoshi; Okuda, Masaki; Ito, Kiyoshi
CORPORATE SOURCE: National Research Institute of Brewing, Higashi-Hiroshima, 739-0046, Japan
SOURCE: Journal of Bioscience and Bioengineering (2000), 90(6), 643-647
CODEN: JBBIF6; ISSN: 1389-1723
PUBLISHER: Society for Bioscience and Bioengineering, Japan
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Kyokai no. 7 is the most widely used yeast in sake brewing. This yeast is a pantothenic acid auxotroph at 35.degree.C, and this phenotype has been used to distinguish Kyokai no. 7 from other sake yeasts. We cloned a DNA fragment complementing the pantothenic acid auxotrophy from a genomic library of a Saccharomyces cerevisiae lab. strain. DNA sequence anal. revealed that the DNA fragment encodes ECM31, the **deletion** of which had previously been identified as a calcofluor white-sensitive mutation. The ECM31 product is similar to the Escherichia coli **ketopantoate hydroxymethyltransferase**. Disruption of ECM31 in a lab. S. cerevisiae strain resulted in pantothenic acid auxotrophy, indicating that ECM31 is also involved in pantothenic acid synthesis in yeast. A hybrid of a Kyokai no. 7 haploid and the ecm31 disruptant required pantothenic acid at 35.degree.C for its growth, suggesting that Kyokai no. 7 possesses a temp.-sensitive allele of ECM31. Thus, the ECM31 gene can be used as a selective marker in the transformation of Kyokai no. 7.

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:593009 HCAPLUS
DOCUMENT NUMBER: 133:176272

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001092556	A1	20011206	WO 2001-EP4816	20010428
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
DE 10026758	A1	20011206	DE 2000-10026758	20000530
EP 1285083	A1	20030226	EP 2001-940381	20010428
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2002042104	A1	20020411	US 2001-852118	20010510

PRIORITY APPLN. INFO.: DE 2000-10026758 A 20000530
WO 2001-EP4816 W 20010428

AB The invention provides a process for prepg. D-pantothenic acid by the fermn. of coryneform bacteria in which bacteria are used in which the nucleotide sequence (pck gene) coding for phosphoenolpyruvate carboxykinase (EC 4.1.1.49) is attenuated, wherein the following steps are performed: fermn. of D-pantothenic acid producing bacteria in which at least the gene coding for phosphoenolpyruvate carboxykinase is attenuated, enrichment of D-pantothenic acid in the medium or in the cells of the bacteria, and isolation of the D-pantothenic acid produced.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 3 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:169488 HCAPLUS
DOCUMENT NUMBER: 135:252592
TITLE: Molecular cloning and application of a gene complementing pantothenic acid auxotrophy of sake yeast Kyokai no. 7
AUTHOR(S): Shimoi, Hitoshi; Okuda, Masaki; Ito, Kiyoshi
CORPORATE SOURCE: National Research Institute of Brewing, Higashi-Hiroshima, 739-0046, Japan
SOURCE: Journal of Bioscience and Bioengineering (2000), 90(6), 643-647
CODEN: JBBIF6; ISSN: 1389-1723
PUBLISHER: Society for Bioscience and Bioengineering, Japan
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Kyokai no. 7 is the most widely used yeast in sake brewing. This yeast is a pantothenic acid auxotroph at 35.degree.C, and this phenotype has been used to distinguish Kyokai no. 7 from other sake yeasts. We cloned a DNA fragment complementing the pantothenic acid auxotrophy from a genomic library of a Saccharomyces cerevisiae lab. strain. DNA sequence anal. revealed that the DNA fragment encodes ECM31, the **deletion** of which had previously been identified as a calcofluor white-sensitive mutation. The ECM31 product is similar to the Escherichia coli **ketopantoate hydroxymethyltransferase**. Disruption of ECM31 in a lab. S. cerevisiae strain resulted in pantothenic acid auxotrophy, indicating that ECM31 is also involved in pantothenic acid synthesis in yeast. A hybrid of a Kyokai no. 7 haploid and the ecm31 disruptant required pantothenic acid at 35.degree.C for its growth, suggesting that Kyokai no. 7 possesses a temp.-sensitive allele of ECM31. Thus, the ECM31 gene can be used as a selective marker in the transformation of Kyokai no. 7.

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:593009 HCAPLUS
DOCUMENT NUMBER: 133:176272

TITLE: Manufacture of L-valine with microbial hosts overexpressing genes of valine biosynthesis
INVENTOR(S): Eggeling, Lothar; Sahm, Hermann
PATENT ASSIGNEE(S): Forschungszentrum Juelich G.m.b.H., Germany
SOURCE: Ger. Offen., 24 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 19907567	A1	20000824	DE 1999-19907567	19990222
WO 2000050624	A1	20000831	WO 2000-EP1405	20000221
W: CZ, JP, KR, SK, US, ZA				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 1155139	A1	20011121	EP 2000-906363	20000221
EP 1155139	B1	20030409		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002537771	T2	20021112	JP 2000-601187	20000221
AT 236991	E	20030415	AT 2000-906363	20000221

PRIORITY APPLN. INFO.: DE 1999-19907567 A 19990222
WO 2000-EP1405 W 20000221

AB A method of increasing the yield of L-valine in microbial fermn. by altering activities of enzymes of valine biosynthesis or levels of gene expression is described. The ilvD gene encoding the dihydroxyacid dehydratase is the most important. Alternatively, or in addn., the ilvBN gene for acetohydroxyacid synthase and the ilvC (isomeroreductase) can also be manipulated to increase activity or gene expression. At the same time, genes that may limit valine biosynthesis, notably the ilvA gene for threonine dehydratase, is inactivated. Similarly, the mutation may also be in the genes for the enzymes of pantothenate biosynthesis. Deletion of the ilvA and panBC genes and increasing the expression of the ilvBNCD cluster increased the yield of valine from 0.5 mM for the parental strain to to 72.7 mM.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:628166 HCAPLUS
DOCUMENT NUMBER: 132:19394
TITLE: The Aspergillus nidulans panB gene encodes ketopantoate hydroxymethyltransferase, required for biosynthesis of pantothenate and Coenzyme A
AUTHOR(S): Kurtov, D.; Kinghorn, J. R.; Unkles, S. E.
CORPORATE SOURCE: Department of Microbiology, Monash University, Clayton, 3168, Australia
SOURCE: Molecular and General Genetics (1999), 262(1), 115-120
CODEN: MGGEAE; ISSN: 0026-8925
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Ketopantoate hydroxymethyltransferase**, which is encoded by the panB gene in the lower eukaryote Aspergillus nidulans, is essential for the biosynthesis of CoA, while the pathway intermediate 4'-phosphopantetheine is required for penicillin prodn. **Ketopantoate hydroxymethyltransferase** could also serve as a target for anti-fungal drugs, since it is not present in mammals. Clones of panB were identified by complementation of the corresponding mutant, and the DNA sequence of the gene was detd. The fungal panB gene encodes a predicted protein of mol. mass 37.7 kDa, contg. two short sequence motifs, LeuValGlyAspSer and GlyIleGlyAlaGly, that are completely conserved between prokaryotic and eukaryotic homologues. The mutation panB100 was found to result in **deletion** of Gly-168, the last glycine within the latter conserved motif. Anal. by gel filtration suggests that the fungal PanB protein can be expressed in Escherichia coli

TITLE: Manufacture of L-valine with microbial hosts overexpressing genes of valine biosynthesis
INVENTOR(S): Eggeling, Lothar; Sahm, Hermann
PATENT ASSIGNEE(S): Forschungszentrum Juelich G.m.b.H., Germany
SOURCE: Ger. Offen., 24 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 19907567	A1	20000824	DE 1999-19907567	19990222
WO 2000050624	A1	20000831	WO 2000-EP1405	20000221
W: CZ, JP, KR, SK, US, ZA				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 1155139	A1	20011121	EP 2000-906363	20000221
EP 1155139	B1	20030409		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002537771	T2	20021112	JP 2000-601187	20000221
AT 236991	E	20030415	AT 2000-906363	20000221

PRIORITY APPLN. INFO.: DE 1999-19907567 A 19990222
WO 2000-EP1405 W 20000221

AB A method of increasing the yield of L-valine in microbial fermn. by altering activities of enzymes of valine biosynthesis or levels of gene expression is described. The ilvD gene encoding the dihydroxyacid dehydratase is the most important. Alternatively, or in addn., the ilvBN gene for acetohydroxyacid synthase and the ilvC (isomeroreductase) can also be manipulated to increase activity or gene expression. At the same time, genes that may limit valine biosynthesis, notably the ilvA gene for threonine dehydratase, is inactivated. Similarly, the mutation may also be in the genes for the enzymes of pantothenate biosynthesis. Deletion of the ilvA and panBC genes and increasing the expression of the ilvBNCD cluster increased the yield of valine from 0.5 mM for the parental strain to to 72.7 mM.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:628166 HCAPLUS
DOCUMENT NUMBER: 132:19394
TITLE: The Aspergillus nidulans panB gene encodes ketopantoate hydroxymethyltransferase, required for biosynthesis of pantothenate and Coenzyme A
AUTHOR(S): Kurtov, D.; Kinghorn, J. R.; Unkles, S. E.
CORPORATE SOURCE: Department of Microbiology, Monash University, Clayton, 3168, Australia
SOURCE: Molecular and General Genetics (1999), 262(1), 115-120
CODEN: MGGEAE; ISSN: 0026-8925
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Ketopantoate hydroxymethyltransferase**, which is encoded by the panB gene in the lower eukaryote Aspergillus nidulans, is essential for the biosynthesis of CoA, while the pathway intermediate 4'-phosphopantetheine is required for penicillin prodn. **Ketopantoate hydroxymethyltransferase** could also serve as a target for anti-fungal drugs, since it is not present in mammals. Clones of panB were identified by complementation of the corresponding mutant, and the DNA sequence of the gene was detd. The fungal panB gene encodes a predicted protein of mol. mass 37.7 kDa, contg. two short sequence motifs, LeuValGlyAspSer and GlyIleGlyAlaGly, that are completely conserved between prokaryotic and eukaryotic homologues. The mutation panB100 was found to result in **deletion** of Gly-168, the last glycine within the latter conserved motif. Anal. by gel filtration suggests that the fungal PanB protein can be expressed in Escherichia coli

as an active octameric enzyme. The panB transcript is present in low abundance and, most probably, a small increase in transcript levels occurs in the absence of exogenous pantothenate.

REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:302513 HCAPLUS
DOCUMENT NUMBER: 131:85307
TITLE: D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overproduction
AUTHOR(S): Sahm, Hermann; Eggeling, Lothar
CORPORATE SOURCE: Institut fur Biotechnologie, Forschungszentrum Julich GmbH, Julich, 52425, Germany
SOURCE: Applied and Environmental Microbiology (1999), 65(5), 1973-1979
CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB D-Pantothenate is synthesized via four enzymes from ketoisovalerate, which is an intermediate of branched-chain amino acid synthesis. We quantified three of these enzyme activities in Corynebacterium glutamicum and detd. specific activities ranging from 0.00014 to 0.001 .mu.mol/min mg (protein)-1. The genes encoding the **ketopantoate hydroxymethyltransferase** and the pantothenate synthetase were cloned, sequenced, and functionally characterized. These studies suggest that panBC constitutes an operon. By using panC, an assay system was developed to quantify D-pantothenate. The wild type of C. glutamicum was found to accumulate 9 .mu.g of this vitamin per L. A strain was constructed (i) to abolish L-isoleucine synthesis, (ii) to result in increased ketoisovalerate formation, and (iii) to enable its further conversion to D-pantothenate. The best resulting strain has ilvA **deleted** from its chromosome and has two plasmids to overexpress genes of ketoisovalerate (ilvBNCD) and D-pantothenate (panBC) synthesis. With this strain a D-pantothenate accumulation of up to 1 g/L is achieved, which is a 105-fold increase in concn. compared to that of the original wild-type strain. From the series of strains analyzed it follows that an increased ketoisovalerate availability is mandatory to direct the metabolite flux into the D-pantothenate-specific part of the pathway and that the availability of .beta.-alanine is essential for D-pantothenate formation.

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 7 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:195478 HCAPLUS
DOCUMENT NUMBER: 130:249283
TITLE: Genetic and physiological analysis of the formation of pantothenate and valine in Corynebacterium glutamicum
AUTHOR(S): Reuter, Uwe
CORPORATE SOURCE: Inst. Biotechnologie, Forschungszentrum Juelich G.m.b.H., Juelich, D-52425, Germany
SOURCE: Berichte des Forschungszentrums Juelich (1998), Juel-3606, 1-115 pp.
CODEN: FJBEE5; ISSN: 0366-0885
DOCUMENT TYPE: Report
LANGUAGE: German

AB The Gram-pos. bacterium C. glutamicum is used for the prodn. of amino acids, e.g. of L-glutamate and L-Lys. The biosynthetic pathway of pantothenic acid of this organism was elucidated, and the formation of L-Val and D-pantothenic acid to enable a microbiol. prodn. of these compds was increased. The genes panB and panC were cloned, which encode **ketopantoate hydroxymethyltransferase** and pantothenate synthetase. The 2 enzymes catalyze important steps of the biosynthetic pathway of pantothenate. Sequence anal. revealed that panB comprises 813 bp and panC 837 bp. The genes are organized as an operon. Assays for the enzymes of the pathway were developed. The pantothenate synthetase has a

as an active octameric enzyme. The panB transcript is present in low abundance and, most probably, a small increase in transcript levels occurs in the absence of exogenous pantothenate.

REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:302513 HCAPLUS
DOCUMENT NUMBER: 131:85307
TITLE: D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overproduction
AUTHOR(S): Sahm, Hermann; Eggeling, Lothar
CORPORATE SOURCE: Institut fur Biotechnologie, Forschungszentrum Julich GmbH, Julich, 52425, Germany
SOURCE: Applied and Environmental Microbiology (1999), 65(5), 1973-1979
CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB D-Pantothenate is synthesized via four enzymes from ketoisovalerate, which is an intermediate of branched-chain amino acid synthesis. We quantified three of these enzyme activities in Corynebacterium glutamicum and detd. specific activities ranging from 0.00014 to 0.001 .mu.mol/min mg (protein)-1. The genes encoding the **ketopantoate hydroxymethyltransferase** and the pantothenate synthetase were cloned, sequenced, and functionally characterized. These studies suggest that panBC constitutes an operon. By using panC, an assay system was developed to quantify D-pantothenate. The wild type of C. glutamicum was found to accumulate 9 .mu.g of this vitamin per L. A strain was constructed (i) to abolish L-isoleucine synthesis, (ii) to result in increased ketoisovalerate formation, and (iii) to enable its further conversion to D-pantothenate. The best resulting strain has ilvA **deleted** from its chromosome and has two plasmids to overexpress genes of ketoisovalerate (ilvBNCD) and D-pantothenate (panBC) synthesis. With this strain a D-pantothenate accumulation of up to 1 g/L is achieved, which is a 105-fold increase in concn. compared to that of the original wild-type strain. From the series of strains analyzed it follows that an increased ketoisovalerate availability is mandatory to direct the metabolite flux into the D-pantothenate-specific part of the pathway and that the availability of .beta.-alanine is essential for D-pantothenate formation.

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 7 OF 7 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:195478 HCAPLUS
DOCUMENT NUMBER: 130:249283
TITLE: Genetic and physiological analysis of the formation of pantothenate and valine in Corynebacterium glutamicum
AUTHOR(S): Reuter, Uwe
CORPORATE SOURCE: Inst. Biotechnologie, Forschungszentrum Juelich G.m.b.H., Juelich, D-52425, Germany
SOURCE: Berichte des Forschungszentrums Juelich (1998), Juel-3606, 1-115 pp.
CODEN: FJBEE5; ISSN: 0366-0885
DOCUMENT TYPE: Report
LANGUAGE: German

AB The Gram-pos. bacterium C. glutamicum is used for the prodn. of amino acids, e.g. of L-glutamate and L-Lys. The biosynthetic pathway of pantothenic acid of this organism was elucidated, and the formation of L-Val and D-pantothenic acid to enable a microbiol. prodn. of these compds was increased. The genes panB and panC were cloned, which encode **ketopantoate hydroxymethyltransferase** and pantothenate synthetase. The 2 enzymes catalyze important steps of the biosynthetic pathway of pantothenate. Sequence anal. revealed that panB comprises 813 bp and panC 837 bp. The genes are organized as an operon. Assays for the enzymes of the pathway were developed. The pantothenate synthetase has a

sp. activity of 1 nmol/min-mg protein, **ketopantoate hydroxymethyltransferase** one of 0.14 nmol/min-mg protein, and the aspartate .alpha.-decarboxylase one of 0.11 nmol/min-mg protein. The quant. anal. of the formation of pantothenic acid revealed that C. glutamicum accumulates 10 .mu.g pantothenic acid/L. A system to isolate mutants with an increased formation of pantothenate, which is based on a deficiency of pantothenic acid induced by .alpha.-ketobutyrate, was established. The application of this method lad to the isolation of a mutant which accumulates 250 .mu.g pantothenate and 1.4 g valine/L. Overexpression of the genes of the valine and isoleucine biosynthetic pathway (ilvBNCD), in combination with the **deletion** of the threonine dehydratase gene ilvA, resulted in the construction of a strain which accumulates 11.3 g valine and 190 mg pantothenate/L. Addnl. overexpression of panBC led to an accumulation of .ltoreq.1 g pantothenic acid/L. Thus, an increase of the formation of pantothenic acid in C. glutamicum by a factor of 105 has been achieved.

REFERENCE COUNT: 163 THERE ARE 163 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:886541 HCAPLUS
DOCUMENT NUMBER: 136:36479
TITLE: A process for the fermentative preparation of D-pantothenic acid using coryneform bacteria with deleted pck (phosphoenolpyruvate carboxykinase, 4.1.1.49) gene
INVENTOR(S): Dusch, Nicole; Thierbach, Georg
PATENT ASSIGNEE(S): Degussa Ag, Germany
SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001092556	A1	20011206	WO 2001-EP4816	20010428
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
DE 10026758	A1	20011206	DE 2000-10026758	20000530
EP 1285083	A1	20030226	EP 2001-940381	20010428
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2002042104	A1	20020411	US 2001-852118	20010510

PRIORITY APPLN. INFO.: DE 2000-10026758 A 20000530
WO 2001-EP4816 W 20010428

AB The invention provides a process for prepg. D-pantothenic acid by the fermn. of coryneform bacteria in which bacteria are used in which the nucleotide sequence (pck gene) coding for phosphoenolpyruvate carboxykinase (EC 4.1.1.49) is attenuated, wherein the following steps are performed: fermn. of D-pantothenic acid producing bacteria in which at least the gene coding for phosphoenolpyruvate carboxykinase is attenuated, enrichment of D-pantothenic acid in the medium or in the cells of the bacteria, and isolation of the D-pantothenic acid produced.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

sp. activity of 1 nmol/min-mg protein, **ketopantoate hydroxymethyltransferase** one of 0.14 nmol/min-mg protein, and the aspartate .alpha.-decarboxylase one of 0.11 nmol/min-mg protein. The quant. anal. of the formation of pantothenic acid revealed that C. glutamicum accumulates 10 .mu.g pantothenic acid/L. A system to isolate mutants with an increased formation of pantothenate, which is based on a deficiency of pantothenic acid induced by .alpha.-ketobutyrate, was established. The application of this method lad to the isolation of a mutant which accumulates 250 .mu.g pantothenate and 1.4 g valine/L. Overexpression of the genes of the valine and isoleucine biosynthetic pathway (ilvBNCD), in combination with the **deletion** of the threonine dehydratase gene ilvA, resulted in the construction of a strain which accumulates 11.3 g valine and 190 mg pantothenate/L. Addnl. overexpression of panBC led to an accumulation of .ltoreq.1 g pantothenic acid/L. Thus, an increase of the formation of pantothenic acid in C. glutamicum by a factor of 105 has been achieved.

REFERENCE COUNT: 163 THERE ARE 163 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:886541 HCAPLUS
DOCUMENT NUMBER: 136:36479
TITLE: A process for the fermentative preparation of D-pantothenic acid using coryneform bacteria with deleted pck (phosphoenolpyruvate carboxykinase, 4.1.1.49) gene
INVENTOR(S): Dusch, Nicole; Thierbach, Georg
PATENT ASSIGNEE(S): Degussa Ag, Germany
SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001092556	A1	20011206	WO 2001-EP4816	20010428
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
DE 10026758	A1	20011206	DE 2000-10026758	20000530
EP 1285083	A1	20030226	EP 2001-940381	20010428
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2002042104	A1	20020411	US 2001-852118	20010510

PRIORITY APPLN. INFO.: DE 2000-10026758 A 20000530
WO 2001-EP4816 W 20010428

AB The invention provides a process for prepg. D-pantothenic acid by the fermn. of coryneform bacteria in which bacteria are used in which the nucleotide sequence (pck gene) coding for phosphoenolpyruvate carboxykinase (EC 4.1.1.49) is attenuated, wherein the following steps are performed: fermn. of D-pantothenic acid producing bacteria in which at least the gene coding for phosphoenolpyruvate carboxykinase is attenuated, enrichment of D-pantothenic acid in the medium or in the cells of the bacteria, and isolation of the D-pantothenic acid produced.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:593009 HCAPLUS
DOCUMENT NUMBER: 133:176272
TITLE: Manufacture of L-valine with microbial hosts overexpressing genes of valine biosynthesis
INVENTOR(S): Eggeling, Lothar; Sahm, Hermann
PATENT ASSIGNEE(S): Forschungszentrum Juelich G.m.b.H., Germany
SOURCE: Ger. Offen., 24 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 19907567	A1	20000824	DE 1999-19907567	19990222
WO 2000050624	A1	20000831	WO 2000-EP1405	20000221
W: CZ, JP, KR, SK, US, ZA				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 1155139	A1	20011121	EP 2000-906363	20000221
EP 1155139	B1	20030409		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002537771	T2	20021112	JP 2000-601187	20000221
AT 236991	E	20030415	AT 2000-906363	20000221

PRIORITY APPLN. INFO.: DE 1999-19907567 A 19990222
WO 2000-EP1405 W 20000221

AB A method of increasing the yield of L-valine in microbial fermn. by altering activities of enzymes of valine biosynthesis or levels of gene expression is described. The ilvD gene encoding the dihydroxyacid dehydratase is the most important. Alternatively, or in addn., the ilvBN gene for acetohydroxyacid synthase and the ilvC (isomeroreductase) can also be manipulated to increase activity or gene expression. At the same time, genes that may limit valine biosynthesis, notably the ilvA gene for threonine dehydratase, is inactivated. Similarly, the mutation may also be in the genes for the enzymes of pantothenate biosynthesis. Deletion of the ilvA and panBC genes and increasing the expression of the ilvBNCD cluster increased the yield of valine from 0.5 mM for the parental strain to to 72.7 mM.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:302513 HCAPLUS
DOCUMENT NUMBER: 131:85307
TITLE: D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overproduction
AUTHOR(S): Sahm, Hermann; Eggeling, Lothar
CORPORATE SOURCE: Institut fur Biotechnologie, Forschungszentrum Julich GmbH, Julich, 52425, Germany
SOURCE: Applied and Environmental Microbiology (1999), 65(5), 1973-1979
CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB D-Pantothenate is synthesized via four enzymes from ketoisovalerate, which is an intermediate of branched-chain amino acid synthesis. We quantified three of these enzyme activities in Corynebacterium glutamicum and detd. specific activities ranging from 0.00014 to 0.001 .mu.mol/min mg (protein)-1. The genes encoding the ketopantoate hydroxymethyltransferase and the **pantothenate synthetase** were cloned, sequenced, and functionally characterized. These studies suggest that panBC constitutes an operon. By using panC, an assay system was developed to quantify D-pantothenate. The wild type of C. glutamicum was found to accumulate 9 .mu.g of this vitamin per L. A strain was constructed (i) to

L14 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:593009 HCAPLUS
DOCUMENT NUMBER: 133:176272
TITLE: Manufacture of L-valine with microbial hosts overexpressing genes of valine biosynthesis
INVENTOR(S): Eggeling, Lothar; Sahm, Hermann
PATENT ASSIGNEE(S): Forschungszentrum Juelich G.m.b.H., Germany
SOURCE: Ger. Offen., 24 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 19907567	A1	20000824	DE 1999-19907567	19990222
WO 2000050624	A1	20000831	WO 2000-EP1405	20000221
W: CZ, JP, KR, SK, US, ZA				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 1155139	A1	20011121	EP 2000-906363	20000221
EP 1155139	B1	20030409		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002537771	T2	20021112	JP 2000-601187	20000221
AT 236991	E	20030415	AT 2000-906363	20000221

PRIORITY APPLN. INFO.: DE 1999-19907567 A 19990222
WO 2000-EP1405 W 20000221

AB A method of increasing the yield of L-valine in microbial fermn. by altering activities of enzymes of valine biosynthesis or levels of gene expression is described. The ilvD gene encoding the dihydroxyacid dehydratase is the most important. Alternatively, or in addn., the ilvBN gene for acetohydroxyacid synthase and the ilvC (isomeroreductase) can also be manipulated to increase activity or gene expression. At the same time, genes that may limit valine biosynthesis, notably the ilvA gene for threonine dehydratase, is inactivated. Similarly, the mutation may also be in the genes for the enzymes of pantothenate biosynthesis. Deletion of the ilvA and panBC genes and increasing the expression of the ilvBNCD cluster increased the yield of valine from 0.5 mM for the parental strain to to 72.7 mM.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:302513 HCAPLUS
DOCUMENT NUMBER: 131:85307
TITLE: D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overproduction
AUTHOR(S): Sahm, Hermann; Eggeling, Lothar
CORPORATE SOURCE: Institut fur Biotechnologie, Forschungszentrum Julich GmbH, Julich, 52425, Germany
SOURCE: Applied and Environmental Microbiology (1999), 65(5), 1973-1979
CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB D-Pantothenate is synthesized via four enzymes from ketoisovalerate, which is an intermediate of branched-chain amino acid synthesis. We quantified three of these enzyme activities in Corynebacterium glutamicum and detd. specific activities ranging from 0.00014 to 0.001 .mu.mol/min mg (protein)-1. The genes encoding the ketopantoate hydroxymethyltransferase and the **pantothenate synthetase** were cloned, sequenced, and functionally characterized. These studies suggest that panBC constitutes an operon. By using panC, an assay system was developed to quantify D-pantothenate. The wild type of C. glutamicum was found to accumulate 9 .mu.g of this vitamin per L. A strain was constructed (i) to

abolish L-isoleucine synthesis, (ii) to result in increased ketoisovalerate formation, and (iii) to enable its further conversion to D-pantothenate. The best resulting strain has ilvA **deleted** from its chromosome and has two plasmids to overexpress genes of ketoisovalerate (ilvBNCD) and D-pantothenate (panBC) synthesis. With this strain a D-pantothenate accumulation of up to 1 g/L is achieved, which is a 105-fold increase in concn. compared to that of the original wild-type strain. From the series of strains analyzed it follows that an increased ketoisovalerate availability is mandatory to direct the metabolite flux into the D-pantothenate-specific part of the pathway and that the availability of .beta.-alanine is essential for D-pantothenate formation.

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1999:195478 HCAPLUS

DOCUMENT NUMBER: 130:249283

TITLE: Genetic and physiological analysis of the formation of pantothenate and valine in Corynebacterium glutamicum

AUTHOR(S): Reuter, Uwe

CORPORATE SOURCE: Inst. Biotechnologie, Forschungszentrum Juelich G.m.b.H., Juelich, D-52425, Germany

SOURCE: Berichte des Forschungszentrums Juelich (1998), Juel-3606, 1-115 pp.
CODEN: FJBEE5; ISSN: 0366-0885

DOCUMENT TYPE: Report

LANGUAGE: German

AB The Gram-pos. bacterium C. glutamicum is used for the prodn. of amino acids, e.g. of L-glutamate and L-Lys. The biosynthetic pathway of pantothenic acid of this organism was elucidated, and the formation of L-Val and D-pantothenic acid to enable a microbiol. prodn. of these compds was increased. The genes panB and panC were cloned, which encode ketopantoate hydroxymethyltransferase and **pantothenate synthetase**. The 2 enzymes catalyze important steps of the biosynthetic pathway of pantothenate. Sequence anal. revealed that panB comprises 813 bp and panC 837 bp. The genes are organized as an operon. Assays for the enzymes of the pathway were developed. The **pantothenate synthetase** has a sp. activity of 1 nmol/min-mg protein, ketopantoate hydroxymethyltransferase one of 0.14 nmol/min-mg protein, and the aspartate .alpha.-decarboxylase one of 0.11 nmol/min-mg protein. The quant. anal. of the formation of pantothenic acid revealed that C. glutamicum accumulates 10 .mu.g pantothenic acid/L. A system to isolate mutants with an increased formation of pantothenate, which is based on a deficiency of pantothenic acid induced by .alpha.-ketobutyrate, was established. The application of this method lad to the isolation of a mutant which accumulates 250 .mu.g pantothenate and 1.4 g valine/L. Overexpression of the genes of the valine and isoleucine biosynthetic pathway (ilvBNCD), in combination with the **deletion** of the threonine dehydratase gene ilvA, resulted in the construction of a strain which accumulates 11.3 g valine and 190 mg pantothenate/L. Addnl. overexpression of panBC led to an accumulation of .ltoreq.1 g pantothenic acid/L. Thus, an increase of the formation of pantothenic acid in C. glutamicum by a factor of 105 has been achieved.

REFERENCE COUNT: 163 THERE ARE 163 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

abolish L-isoleucine synthesis, (ii) to result in increased ketoisovalerate formation, and (iii) to enable its further conversion to D-pantothenate. The best resulting strain has ilvA **deleted** from its chromosome and has two plasmids to overexpress genes of ketoisovalerate (ilvBNCD) and D-pantothenate (panBC) synthesis. With this strain a D-pantothenate accumulation of up to 1 g/L is achieved, which is a 105-fold increase in concn. compared to that of the original wild-type strain. From the series of strains analyzed it follows that an increased ketoisovalerate availability is mandatory to direct the metabolite flux into the D-pantothenate-specific part of the pathway and that the availability of .beta.-alanine is essential for D-pantothenate formation.

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1999:195478 HCAPLUS

DOCUMENT NUMBER: 130:249283

TITLE: Genetic and physiological analysis of the formation of pantothenate and valine in Corynebacterium glutamicum

AUTHOR(S): Reuter, Uwe

CORPORATE SOURCE: Inst. Biotechnologie, Forschungszentrum Juelich G.m.b.H., Juelich, D-52425, Germany

SOURCE: Berichte des Forschungszentrums Juelich (1998), Juel-3606, 1-115 pp.
CODEN: FJBEE5; ISSN: 0366-0885

DOCUMENT TYPE: Report

LANGUAGE: German

AB The Gram-pos. bacterium C. glutamicum is used for the prodn. of amino acids, e.g. of L-glutamate and L-Lys. The biosynthetic pathway of pantothenic acid of this organism was elucidated, and the formation of L-Val and D-pantothenic acid to enable a microbiol. prodn. of these compds was increased. The genes panB and panC were cloned, which encode ketopantoate hydroxymethyltransferase and **pantothenate synthetase**. The 2 enzymes catalyze important steps of the biosynthetic pathway of pantothenate. Sequence anal. revealed that panB comprises 813 bp and panC 837 bp. The genes are organized as an operon. Assays for the enzymes of the pathway were developed. The **pantothenate synthetase** has a sp. activity of 1 nmol/min-mg protein, ketopantoate hydroxymethyltransferase one of 0.14 nmol/min-mg protein, and the aspartate .alpha.-decarboxylase one of 0.11 nmol/min-mg protein. The quant. anal. of the formation of pantothenic acid revealed that C. glutamicum accumulates 10 .mu.g pantothenic acid/L. A system to isolate mutants with an increased formation of pantothenate, which is based on a deficiency of pantothenic acid induced by .alpha.-ketobutyrate, was established. The application of this method lad to the isolation of a mutant which accumulates 250 .mu.g pantothenate and 1.4 g valine/L. Overexpression of the genes of the valine and isoleucine biosynthetic pathway (ilvBNCD), in combination with the **deletion** of the threonine dehydratase gene ilvA, resulted in the construction of a strain which accumulates 11.3 g valine and 190 mg pantothenate/L. Addnl. overexpression of panBC led to an accumulation of .ltoreq.1 g pantothenic acid/L. Thus, an increase of the formation of pantothenic acid in C. glutamicum by a factor of 105 has been achieved.

REFERENCE COUNT: 163 THERE ARE 163 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

ilvBN

102b Claim 4, 6, 7, 8, 9

L8 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1990:513851 HCAPLUS
DOCUMENT NUMBER: 113:113851
TITLE: Recombinant microorganisms containing an acetohydroxy acid synthase-encoding vector for manufacture of amino acids
INVENTOR(S): Sato, Katsuaki; Yoshino, Eriko; Hashiguchi, Kenichi; Enei, Hitoshi
PATENT ASSIGNEE(S): Ajinomoto Co., Inc., Japan
SOURCE: Eur. Pat. Appl., 11 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
EP 356739	A1	19900307	EP 1989-114207	19890801	<--
EP 356739	B1	19951213			
R: DE, FR, NL					
JP 02042988	A2	19900213	JP 1988-194031	19880803	<--
JP 2748418	B2	19980506			

PRIORITY APPLN. INFO.: JP 1988-194031 19880803

AB Plasmids or phage contg. a gene for an acetohydroxy acid synthase (AHAS) are prepd. Microorganisms transformed with these vectors can be used to manuf. valine, isoleucine, or leucine. The AHAS gene of Brevibacterium lactofermentum was cloned. B. lactofermentum or B. flavum was transformed with the resulting plasmid (pAJ200V3). The transformants produced 7 and 11 g valine/L culture medium, resp.

Brevibacterium = Corynebacterium

L8 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1984:606512 HCAPLUS
DOCUMENT NUMBER: 101:206512
TITLE: A basal unit of valine-sensitive acetolactate synthase of Neurospora crassa
AUTHOR(S): Tanaka, Hiroshi; Kuwana, Homare
CORPORATE SOURCE: Fac. Sci., Kwansei Gakuin Univ., Nishinomiya, 662, Japan
SOURCE: Biochemical and Biophysical Research Communications (**1984**), 123(2), 418-23
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The valine sensitivity as well as the activity of acetolactate synthase of N. crassa was stabilized with 1.2M K2HPO4 buffer during extn. from mitochondria and early stages of purifn.; 20% glycerol plus 5 mM Na pyruvate was included during purifn. on Sephadex G200 gel chromatog. The enzyme was expressed as 4 subunits having mol. wts. of .apprx.500,000, 140,000, 68,000, and 51,000, resp. The 1st and the 3rd species showed valine sensitivity, but the 2nd and the 4th did not. The subunit with a mol. wt. of 68,000 may be the basal unit of valine-sensitive acetolactate synthase of N. crassa.

L10 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:195478 HCAPLUS
DOCUMENT NUMBER: 130:249283
TITLE: Genetic and physiological analysis of the formation of pantothenate and valine in Corynebacterium glutamicum
AUTHOR(S): Reuter, Uwe
CORPORATE SOURCE: Inst. Biotechnologie, Forschungszentrum Juelich G.m.b.H., Juelich, D-52425, Germany
SOURCE: Berichte des Forschungszentrums Juelich (1998), Juel-3606, 1-115 pp.
CODEN: FJBEE5; ISSN: 0366-0885
DOCUMENT TYPE: Report
LANGUAGE: German

AB The Gram-pos. bacterium C. glutamicum is used for the prodn. of amino acids, e.g. of L-glutamate and L-Lys. The biosynthetic pathway of pantothenic acid of this organism was elucidated, and the formation of L-Val and D-pantothenic acid to enable a microbiol. prodn. of these compds was increased. The genes panB and panC were cloned, which encode **ketopantoate hydroxymethyltransferase** and **pantothenate synthetase**. The 2 enzymes catalyze important steps of the biosynthetic pathway of pantothenate. Sequence anal. revealed that panB comprises 813 bp and panC 837 bp. The genes are organized as an operon. Assays for the enzymes of the pathway were developed. The **pantothenate synthetase** has a sp. activity of 1 nmol/min-mg protein, **ketopantoate hydroxymethyltransferase** one of 0.14 nmol/min-mg protein, and the aspartate .alpha.-decarboxylase one of 0.11 nmol/min-mg protein. The quant. anal. of the formation of pantothenic acid revealed that C. glutamicum accumulates 10 .mu.g pantothenic acid/L. A system to isolate mutants with an increased formation of pantothenate, which is based on a deficiency of pantothenic acid induced by .alpha.-ketobutyrate, was established. The application of this method lad to the isolation of a mutant which accumulates 250 .mu.g pantothenate and 1.4 g **valine** /L. Overexpression of the genes of the **valine** and isoleucine biosynthetic pathway (ilvBNCD), in combination with the deletion of the threonine dehydratase gene ilvA, resulted in the construction of a strain which accumulates 11.3 g **valine** and 190 mg pantothenate/L. Addnl. overexpression of panBC led to an accumulation of .ltoreq.1 g pantothenic acid/L. Thus, an increase of the formation of pantothenic acid in C. glutamicum by a factor of 105 has been achieved.

1

ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2003 ACS on STN
ESSION NUMBER: 1996:342147 HCAPLUS
CUMENT NUMBER: 125:4414
ITLE: Cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and its use for manufacturing isoleucine and valine
INVENTOR(S): Inui, Masayuki; Man, Tomoko; Kobayashi, Miki; Yugawa, Hideaki
PATENT ASSIGNEE(S): Mitsubishi Chem Corp, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
INT. PATENT CLASSIF.:
MAIN: C12N015-09
ADDITIONAL: C12N009-88
INDEX: C12N015-09, C12R001-13; C12N009-88, C12R001-13
CLASSIFICATION: 7-2 (Enzymes)
Section cross-reference(s): 10
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 08089249	A2	19960409	JP 1994-234612	19940929

PRIORITY APPLN. INFO.: JP 1994-234612 19940929

ABSTRACT:
The gene encoding dihydroxy-acid dehydratase (E.C. 4.2.1.9) is isolated from Brevibacterium flavum strain MJ-233. Expression plasmid pCRY30-DH encoding the enzyme was prepd. and used for the transformation of coryneform bacteria. Brevibacterium flavum strain MJ-233 transformed with the plasmid produced isoleucine 20 mM into the medium as compared to 10 mM by the wild type.

SUPPL. TERM: coryneform bacteria dihydroxy acid dehydratase gene; valine isoleucine manuf Brevibacterium
INDEX TERM: Brevibacterium flavum
Deoxyribonucleic acid sequences
Protein sequences
(cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and use for manufg. isoleucine and valine)
INDEX TERM: Gene, microbial
ROLE: MSC (Miscellaneous)
(cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and use for manufg. isoleucine and valine)
INDEX TERM: Plasmid and Episome
(pCRY30-DH; expression of gene for dihydroxy-acid dehydratase of Brevibacterium flavum on)
INDEX TERM: Bacteria
(coryneform, cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and use for manufg. isoleucine and valine)
INDEX TERM: 177474-84-9
ROLE: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence; cloning of gene for dihydroxy-acid dehydratase of coryneform bacteria and use for manufg. isoleucine and valine)
INDEX TERM: **72-18-4P**, **Valine**, preparation 73-32-5P, Isoleucine, preparation
ROLE: BPN (Biosynthetic preparation); BIOL (Biological study); **PREP (Preparation)**
(cloning of gene for **dihydroxy-acid dehydratase** of coryneform bacteria and use for manufg. isoleucine and **valine**)
INDEX TERM: 9024-32-2, Dihydroxy-acid dehydratase